Regulation of Acquired Drug Resistance by miRNAs in High Grade Serous Ovarian Cancer

by

Elif Merve Aydın

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Koc University

Graduate School of Health Sciences

This is to certify that I have examined this copy of a doctoral dissertation by

Elif Merve Aydın

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

Assist. Prof. İrem Durmaz Şahin (Advisor)

Prof. Tuğba Bağcı Önder

Prof. Özgür Öktem

Assist. Prof. Zeynep Tokçaer Keskin

Assist. Prof. Dilek Çevik

Date: 23 July 2024

This thesis is dedicated to my family.

ABSTRACT

Regulation of Acquired Drug Resistance by miRNAs in High Grade Serous Ovarian Cancer

Elif Merve Aydın

Doctor of Philosophy in Cellular and Molecular Medicine

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Epithelial ovarian cancer is the seventh most common malignancy diagnosed in women worldwide and the fifth most common cause of cancer-related deaths. Limited improvements in outcomes have been made over the past decades, and the relative five-year survival rate is less than 50%. High-grade serous ovarian cancer (HGSOC) is the predominant histological subtype. PARP inhibitors such as Olaparib (Lynparza™), and niraparib (Zejula™) have been approved for the treatment of platinum sensitive, recurrent HGSOC. However, like many other targeted agents, the efficacy of PARP inhibitors is limited by the development of drug resistance.

miRNAs are small (21-23 nucleotide long) non-coding RNA molecules that play a role in post-translational regulation of gene expression. Several cellular pathways are affected by miRNAs, such as cell differentiation, proliferation, and apoptosis. The aim of this project is to explore the role of miRNAs in the context of treatment response and resistance in high grade serous ovarian cancer. To this end, drug resistant HGSOC cells established with *ex vivo* cultures of primary cells obtained from resistant (patients with poor prognosis after maximal cytoreductive surgery) and sensitive (patients with good prognosis after maximal cytoreductive surgery) patients diagnosed with HGSOC to investigate the underlying mechanisms of drug resistance towards clinically used drugs.

188-5p gene expression level was found significantly downregulated in Olaparib resistant OVCAR-3 cells and resistant FFPE patient samples with poor prognosis after cytoreductive surgery. Combinational treatment studies showed that synergistic effect of Olaparib and *188-5p* mimic decreased the survival rate of the resistant cells. Downstream effects of synergistic treatment of the *188-5p* mimic and Olaparib exhibited an increase in caspase-8 mediated apoptosis levels in resistant cells. Moreover, in cell cycle experiments, subG1 arrest was observed upon *188-5p* transfection. Results revealed that upregulating mir*188-5p* level in resistant cells significantly suppressed the epithelial-to-mesenchymal transition of resistant cells by decreasing N-cadherin and SNAIL and increasing E-cadherin protein levels.

ÖZETÇE

Yüksek Dereceli Seröz Over Kanserinde Ilaç Direncinin Oluşumunda miNAların Etkileri

Elif Merve Aydın

Hücresel ve Moleküler Tıp, Doktora

Temmuz 23, 2024

Epitelyal over kanseri, dünya çapında kadınlarda tanı konulan yedinci en yaygın kanser çeşididir, ancak kanserle ilişkili ölümlerin en yaygın beşinci nedenidir. Son on yılda, sonlanımda sınırlı gelişmeler kaydedilmiştir ve beş yıllık sağkalım %50'den azdır. Yüksek dereceli seröz over kanseri (HGSOC), baskın histolojik alt tiptir. Birinci basamak kemoterapiye ilk cevap oranları iyi olsa da hastaların %15-20'si yanıt vermemektedir (iç direnç denir) ve %25'i 6 ay içinde direnç geliştirir. Olaparib (Lynparza ™), niraparib (Zejula ™) vb gibi PARP inhibitörleri, platine duyarlı, tekrarlayan HGSOC tedavisi için onaylandı. Bununla birlikte, hedeflenen diğer birçok maddeye benzer şekilde, PARP inhibitörlerinin etkinliği, ilaç direncinin gelişimi ile sınırlıdır.

miRNA'lar, gen ekspresyonunun translasyon sonrası regülasyonunda rol oynayan küçük (21-23 nükleotid uzunluğunda) kodlamayan RNA molekülleridir. Birçok kanser türünde, miRNA'nın tümör baskılayıcı gen veya onkogen olarak tümör ilerlemesinde önemli roller oynadığı gösterilmiştir.

Bu projenin amacı, miRNA'ların tedavi yanıtı ve yüksek dereceli seröz over kanserinde direnç bağlamındaki rolünü araştırmaktır. Bu konseptte, ilaca dirençli HGSOC hücreleri, klinik olarak kullanılan ilaçlara karşı altta yatan ilaç direnci mekanizmalarını araştırmak amacıyla, HGSOC ile teşhis edilen kemoterapiye dirençli (maximal sitoredüktif cerrahi yapılmış olgularda kötü seyirli hastalar) ve kemoterapiye cevap veren (maximal sitoredüktif cerrahi yapılmış olgularda iyi seyirli hastalar) hastalardan elde edilen primer hücrelerin *ex vivo* kültürleriyle birlikte oluşturulmuştur.

188-5p'nin olaparib ile kombinasyonal tedavisinin dirençli hücrelerde apoptozu arttırdığı Annexin-V IF ve Hücre akışı deneyleriyle ve protein düzeyinde hem *in vitro* hem *ex vivo* hasta örneklerinde cleaved PARP'ın anlamlı derecede artması ile gösterilmiştir. Cleaved caspase 8'in anlamlı olarak artması ise bu apoptozun extrinsic yolağını kullandığını düşündürmektedir. EMT belirteçleri üzerine olan western sonucu ve yara izi iyileşme deneyleri sonuçları ise sinerjistik birlikteliğin hücrelerin metastaz kapasitesini anlamlı olarak baskıladığını ortaya koymaktadır.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

µg	Microgram
µl	Microliter
µM	Micromolar
7-AAD	7-Aminoctinomycin
AKT	Protein kinase B
Amp B	Amphotericin B
ATR	Ataxia telangiectasia and Rad3 related
BAK	Bcl-2 Antagonist Killer
Bax	Bcl-2-associated X protein
BC	Breast Cancer
BCA	Bicinchoninic acid
BID	BH3 interacting-domain death
BIK	Bcl-2 Interacting Killer
BIM	Bcl-2 Interacting Mediator of cell death
BRCA	BReast CAncer gene
CCNG1	Cyclin G1
CHK1	Checkpoint Kinase 1
CI	Combination index
CO2	Carbon dioxide
DAPI	4’,6-diamino-2-phenylindole
ddH2O	Doubled distilled water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco’s Phosphate Buffered Saline
DRR	DNA Damage Response
E-cadherin	Epithelial-cadherin
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EMT	Epithelial-mesenchymal transition
EOC	Epithelial ovarian cancer

ERK	Extracellular signal-regulated kinase
FBS	Fetal bovine serum
FOXN2	Forkhead Box N2
G	Gram
G0/G1	Gap 0 phase/Gap 1 phase
G2/M	Gap 2 phase/Mitosis
H2AX	Histone family member X
HGSOC	High-grade serous ovarian cancer
HR	Homologous recombination
IC_{50}	Half-maximal inhibitory concentration
IF	Immunofluorescence
kDa	kilodaltons
LARP4	La ribonucleoprotein domain family member 4
MDR	Multidrug resistance
ml	Milliliter
MOMP	Mitochondrial outer membrane permeabilization
N/I	No Inhibiton
NaN_3	Sodium azide
N-cadherin	Neural cadherin
NFKB	Nuclear factor kappa B
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency.
OC	Ovarian cancer
P/S	Penicillin/Streptomycin
P53	Tumor protein 53
p-Akt	Phosphorylated protein kinase B
PARP-1	Poly (ADP-ribose) polymerase 1
PCa	Prostate Cancer
PhosSTOP	Phosphatase inhibitors
PI	Propidium Iodide
PI3K	Phosphoinositide 3-kinase
PIC	Protease inhibitor cocktail
p-p53	Phosphorylated tumor protein 53
PS	Phosphatidlyserine

PVDF	Polyvinylidene fluoride
RAD51D	RAD51 parolog D
Ras	Rat sarcoma virus
Rb	Retinoblastoma
RIPA	Radioimmunoprecipitation assay
RNaseA	Ribonuclease A
Rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RT	Room temperature
S	Seconds
SD	Standard deviation
SDS	Sodium dodeyl sulfate
SRB	Sulforhodamine B
SubG1	Sub G1 phase
TBS	Tris base solution
TBS-T	Tris buffer saline with Tween-20
TCA	Trichloroacetic acid
TGS	Tris-glycine-SDS
TMBIM1	Transmembrane BAX Inhibitor Motif Containing I
V	Volt
XRCC5	X Ray Repair Cross Complementing 5

Chapter 1:
INTRODUCTION

1.1 Epithelial Ovarian Cancer (EOC)

Significant scientific advancements have greatly improved our understanding of the molecular and pathological characteristics of many cancer types, shedding light on the complex nature of EOC (Gubbels et al., 2010. EOC is a significant global public health issue, characterized by a wide range of histopathological features (Zhou et al., 2018). The incidence and clinical outcomes of this malignancy demonstrate notable geographical variations. Epidemiological data indicates that the global distribution of EOC exhibits regional variations, with the highest incidence rates observed in Eastern and Central Europe, while China exhibits the lowest prevalence (Bogani et al., 2017 and Pisani et al., 2001). Furthermore, this malignancy is classified as the 7th most common cancer worldwide (Kim et al., 2016).

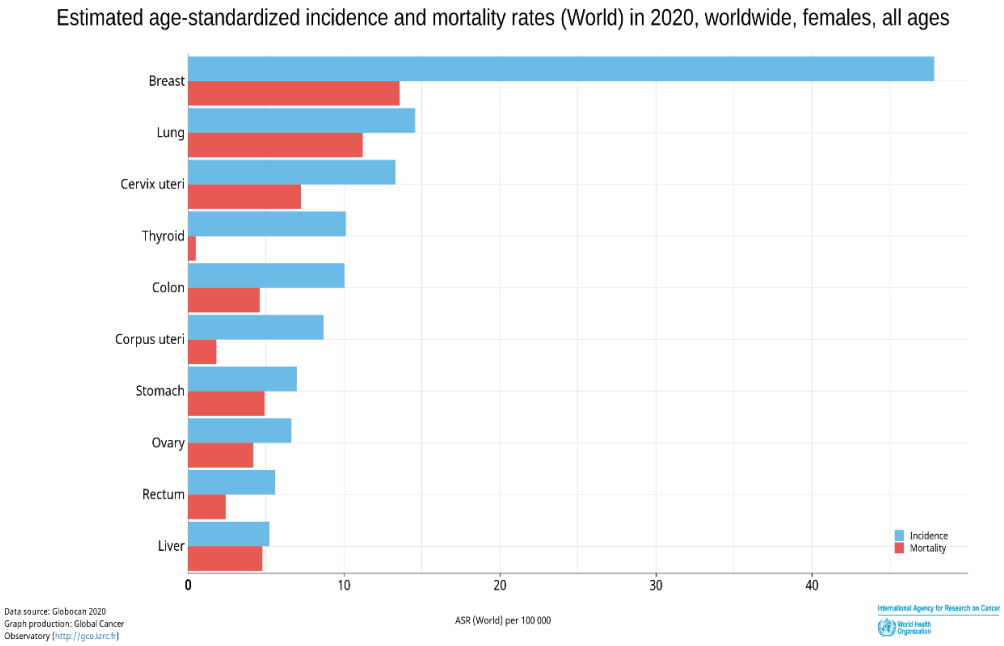


Figure 1.1: Estimated age standardized incidence and mortality rates of EOC, worldwide (Retrieved from data source (GLOBOCAN, 2020)).

1.1.1 Epidemiology of Epithelial Ovarian Cancer

EOC is a leading cause of cancer-related mortality among women, with an average of 150,000 annual deaths reported (Kim et al., 2011). This concerning trend has persisted in recent years, as the number of women diagnosed with ovarian cancer exceeded 300,000 in 2020, leading to approximately 200,000 cancer related deaths (Torre et al., 2018). Despite ongoing research efforts, the treatment approaches for this disease have not shown many improvements. The incidence of EOC is projected to increase markedly by the year 2040, with a predicted doubling in the number of diagnosed patients (Zhang et al., 2016). While treatment outcomes for EOC vary based on the individual patient's clinical presentation, certain risk factors significantly impact progression. Notably, genetic predisposition and hereditary mutations are major risk factors (Bogani et al., 2017). Additionally, patient age plays a crucial role in the development and progression of the disease. Treatment outcomes tend to be less favorable for women diagnosed with OC at age 65 or older (Yancik, 2010).

Unfortunately, the elevated mortality rate for this condition is largely attributed to the limited efficacy of current detection methods, leading to late-stage diagnosis when the cancer has already metastasized. One of the primary factors contributing to this disparate outcome is the tendency for the disease to be diagnosed only in its later stages, as 80% of diagnosed patients present with advanced-stage disease (Hudock et al., 2023). Moreover, the highly heterogeneous genetic profile of the disease, which includes aggressive subtypes, has been a significant factor underlying the high mortality rates. Consequently, the development of novel biomarkers and the exploration of more advanced treatment approaches for patients with late-stage, aggressive forms of OC are crucial for improving outcomes for the lives of many women (Lisio et al., 2019 and Assis et al., 2018).

1.1.2 Origin of Epithelial Ovarian Cancer

In the past, limited research on EOC led to the belief that this disease solely originated from variations in ovarian tissue. However, extensive investigations into the heterogeneous nature of this malignancy have now revealed that it does not exclusively

arise from ovarian epithelial cells but can develop from the entire female reproductive system (Auersperg, 2013). While over 90 percent of the differentiated cells driving this type of cancer are epithelial, other non-epithelial ovarian cancers can arise from sex-cord stromal and germ cells (Chen et al., 2011).

EOC is categorized into two main subtypes, Type I and Type II, based on the cellular origins driving the disease (Gubbels et al., 2010). Type I EOC exhibit a slow progression, arising from ovarian epithelial tissues, and tend to be diagnosed and treated more effectively compared to the Type II. These Type I tumors often harbor mutations in oncogenes such as KRAS, BRAF, and ERBB2, and display a more homogeneous cellular structure than the Type II tumors, which confers a more favorable prognosis for treatment (Kelly, 2014).

In contrast, Type II EOC is characteristically aggressive, rapidly progressing, and more challenging to detect and treat due to their highly heterogeneous cellular composition, frequently exhibiting TP53 and BRCA1/2 genetic mutations (Bergstrom et al., 2017). Individuals diagnosed with the aggressive Type II EOC subtype exhibit unfavorable prognoses and survival outcomes. This category encompasses high-grade serous (HGSOC), endometrioid, clear cell, and mucinous carcinomas, each displaying distinct molecular characteristics and clinical presentations (Cheng et al., 2020). The predominant histological subgroup of Type 2 EOC is HGSOC, comprising approximately 70% of cases (Bowtell, 2012). Significantly, research has demonstrated that in most of these patients, the cancer arises from intraepithelial carcinomas located in the fimbrial region of the fallopian tubes, which facilitate the transport of eggs from the uterus (Auersperg, 2013).

Another proposed mechanism for the development of EOC involves the implantation of normal fimbrial epithelial cells within the ovary during ovulation (Dietl et al., 1993). This

process can lead to the formation of cortical inclusion cysts, which are associated with an increased risk of malignancy (Devouassoux-Shisheboran et al., 2015).

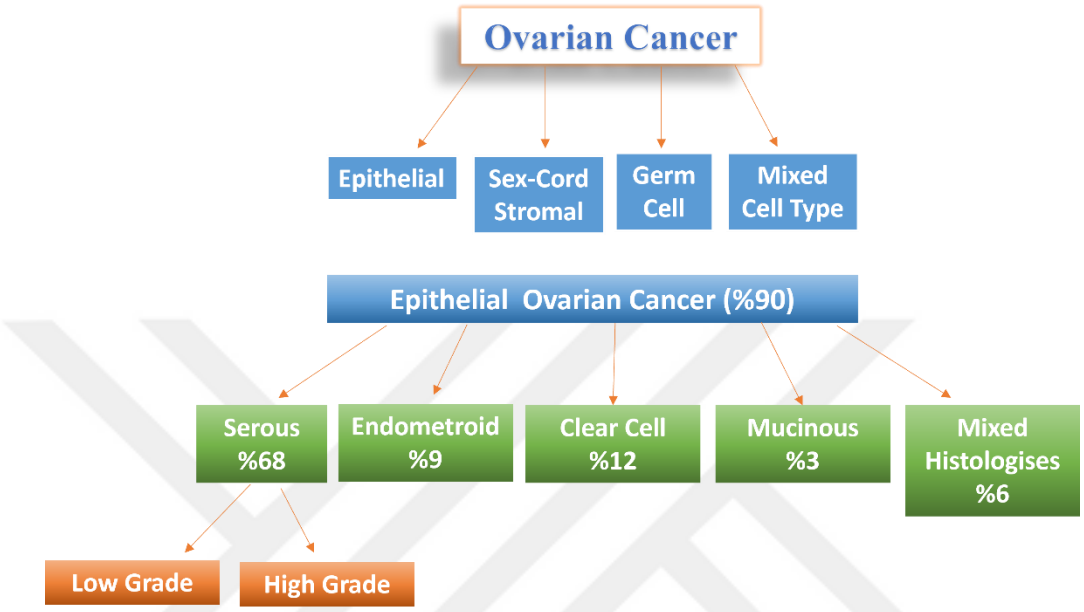


Figure 1.2: Histological subtypes of ovarian cancer; and epithelial ovarian cancer classification based on type I and type II tumors develop through different pathways. (Redrawed from (Kurman et al. 2016)).

These two distinct mechanisms underlie the heterogeneous structure and morphological diversity of the disease. Identifying the source of HGSOC cells and analyzing their genetic mutations is crucial for diagnosing EOC, as it helps better understanding for the disease and develop effective treatment strategies (Bowtell, 2012).

1.2 High Grade Serous Epithelial Ovarian Cancer (HGSOC)

HGSOC represents the most heterogeneous and aggressive subtype of EOC (Rosen et al., 2010). This marked heterogeneity is predominantly driven by the genetic aberrations present within the tumor cells. Most HGSOCs are characterized by TP53 mutations, which serve as a defining feature of this malignancy (Kroeger et al., 2017). Moreover, some tumors exhibit chromosomal instability and gene amplifications. Approximately 30% of patients diagnosed with HGSOC harbor inherited mutations in the BRCA1 or BRCA2 genes, as well as other genes implicated in the homologous recombination DNA

repair (HRR) pathway. These BRCA mutations impair the HR, eventually resulting in defective DNA repair (Pujol et al., 2022).

The Cancer Genome Atlas (TCGA) project has significantly advanced our understanding of the molecular underpinnings of HGSOC. Specifically, the TCGA project has identified some genes and pathways that are highly expressed in advanced-stage OC patients who experienced prolonged disease-free survival of at least 5 years following initial treatment. TCGA project revealed the dysregulation of key oncogenic pathways, including PI3K/AKT, RAS/MAPK, and Notch signaling, in the development and progression of HGSOC (Zhang et al., 2020). Additionally, the overexpression and activation of the MET/HGF signaling cascade has emerged as a critical driver (Wang et al., 2022). The secretion of hepatocyte growth factor (HGF) by cancer-associated fibroblasts can stimulate the activation of the MET receptor on OC cells, consequently enhancing their proliferation, metastatic potential, and angiogenesis (Mhawech-Fauceglia et al., 2012). Furthermore, previous studies have revealed that the high-mobility group A1 (HMGA1) transcription factor, which is commonly overexpressed in HGSOC, can regulate the expression of genes associated with Cancer Stem Cell (CSC) characteristics, including KLF4, SOX2, and ABCG2 (Xi et al., 2014).

Emerging evidence indicates that the molecular heterogeneity observed in EOC can substantially impact their responsiveness to distinct therapeutic strategies (Wang et al., 2022). For example, HGSOC characterized by heightened genomic instability may exhibit increased sensitivity to DNA-damaging agents like platinum-based compounds (Martin et al., 2008). Conversely, clear cell and endometrioid subtypes frequently display alterations in the PI3K/AKT/mTOR signaling pathway, potentially rendering them more vulnerable to targeted therapies that inhibit this critical signaling cascade (Brader et al., 2004).

1.3 Treatment Modalities of HGSOC

Patients diagnosed with HGSOC who are not in the late stages and are suitable candidates undergo cytoreductive surgery as a first step of treatment after evaluation by oncologists and pathologists. For patients whose condition precludes surgery, the goal is to improve the tumor's advance through neoadjuvant chemotherapy prior to surgery (Weinberg et al.,

2010). After cytoreductive surgery, the primary treatment strategy involves administering platinum-based chemotherapeutic agents. Platinum-based chemotherapeutic agents commonly used for the treatment of HGSOC are carboplatin and cisplatin. In certain cases, these drugs are combined with taxol (Tomas et al., 2023). Platinum-based drugs, such as carboplatin and cisplatin, directly bind to and impair the DNA structure through the formation of DNA adducts, thereby inhibiting transcription and arresting cell division (Sousa et al., 2014).

While this class of agents generally demonstrates efficacy, they may elicit critical adverse effects, such as kidney dysfunction and neuropathy (Lameire, 2013). In contrast, taxane-based chemotherapeutic drugs operate through distinct mechanisms, yet both groups ultimately aim to induce cancer cell death by disrupting cellular division. Specifically, taxol or paclitaxel achieves this by binding to and destabilizing microtubules, leading to a stabilized microtubule configuration and cell cycle arrest. Paclitaxel, broadly utilized in ovarian, breast, and lung cancers, additionally carries a serious adverse effect of inducing abnormal microtubule bundling (Abal et al., 2003).

Despite the serious side effects, the main obstacle to effective first-line chemotherapy is chemotherapy resistance. While initial response rates are good, 15-20% of patients do not respond, and 25% develop resistance within 6 months (Hait et al., 2007). This concerning recurrence rate is a major contributor to the high mortality of HGSOC (Vasey, 2003).

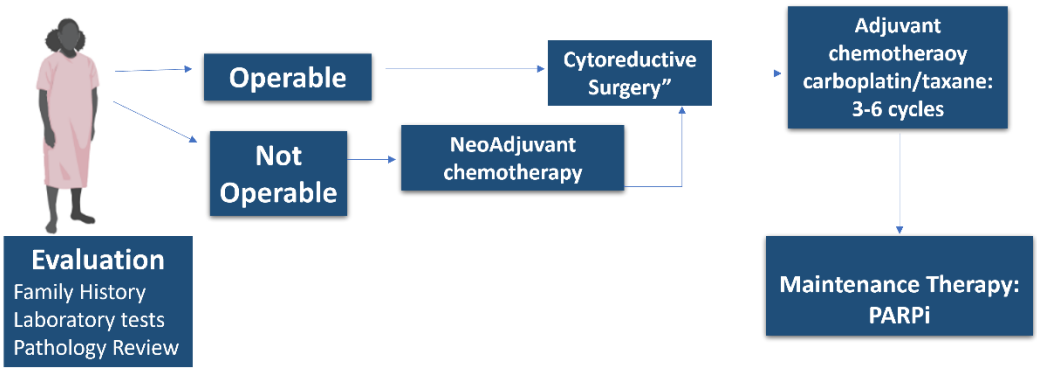


Figure 1.3: Treatment Modalities of Epithelial Ovarian Cancer

1.3.1 Second Line Maintenance Therapy with the poly (ADP-ribose) polymerase (PARP) Inhibitors

PARP family consists of 17 members, with PARP1 being the most extensively studied. This family plays crucial roles in various cellular processes, including telomere maintenance and Wnt signaling, but its most essential function is in DNA repair, where PARP1 is a key component. PARP-1 is a critical component in the cellular response to DNA damage, and its inhibition has demonstrated therapeutic potential for the management of diverse cancer types, including HGSOC (Morales et al., 2014).

Cells have six fundamental DNA repair mechanisms to protect against DNA damage and maintaining genomic integrity (Kennedy et al., 2006). (1) Direct Reversal Repair (DRR) directly corrects DNA damage without the need for DNA synthesis, utilizing agents like O6-methylguanine-DNA methyltransferase and alkylated DNA repair protein B homologues (Yi et al., 2013). (2) The Mismatch Repair (MMR) mechanism ensures accurate replication by correcting mismatched bases (Mishina et al., 2006). (3) The Nucleotide Excision Repair (NER) Mechanism safeguards the DNA helix against damaging agents like UV rays, by excising and resynthesizing the damaged strand (Spivak, 2015). (4) The Base Excision Repair (BER) mechanism aims to rectify damaged bases without disrupting the DNA helix, with PARP1 playing a crucial role in activating this process (Zharkov et al., 2008). Additionally, (5) Homologous (HR) and (6) Non-Homologous End Joining (NHEJ) repair mechanisms are engaged when base excision repair is unavailable or in the case of double-stranded DNA damage. Proper functioning of these mechanisms is essential to prevent serious cellular consequences and cell death (Daley et al., 2013). In a cell with single-stranded DNA damage, inhibition of PARP1 function using PARP inhibitors leads to the occurrence of double-stranded DNA damage. When PARP is inhibited in a cell lacking the HR gene, as seen in HGSOC, DDR mechanisms are unable to fulfill their function, resulting in cell death (McCann et al., 2019).

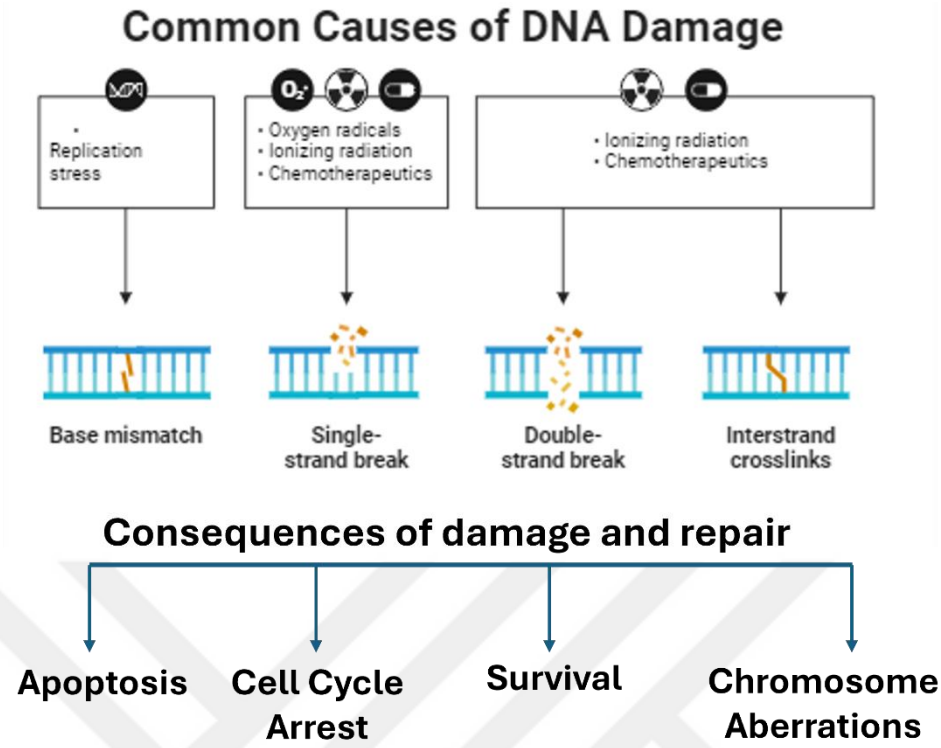


Figure 1.4: Common DNA damaging agents, DNA Repair Mechanisms and consequences. *Note.* Re-drawed from "Cytotoxicity and Genotoxicity Reporter Systems Based on the Use of Mammalian Cells" by Crista et al., 2010.

Olaparib, niraparib, and rucaparib are PARP inhibitors that have received approval for the second-line treatment of platinum-sensitive, relapsed HGSOC. Initially, these PARP inhibitors were only used for patients with BRCA 1/2 mutations, but their use has since been expanded to all individuals with HGSOC (Bitler et al., 2017). Despite the significant therapeutic advancement that PARP inhibitors such as Olaparib have represented in the treatment of HGSOC, the emergence of acquired resistance remains a major obstacle in enhancing patient survival outcomes. The precise mechanisms prompting the development of olaparib resistance remain complex and require further investigation (Biegała et al., 2023).

1.4 Resistant Mechanisms in HGSOC

Patients may exhibit two distinct types of resistance to chemotherapeutic agents: acquired resistance and intrinsic resistance. As described in the literature, acquired resistance emerges over the course of treatment, as the tumor cells progressively develop mechanisms to mitigate the cytotoxic effects of the therapeutic agents (Chan et al., 2012).

Patients who initially respond well to treatment may subsequently develop acquired resistance, wherein the tumor cells acquire mechanisms to diminish the cytotoxic effects of the therapeutic agents, leading to disease relapse and unresponsiveness to continues therapeutic approaches (Shi et al., 2023).

Tumors that exhibit intrinsic resistance are non-responsive to initial therapeutic intervention, as this form of resistance is already established by the time of diagnosis (English et al., 2016). The primary difference between acquired resistance and intrinsic resistance is that acquired resistance arises over the course of treatment, as the tumor cells gradually evolve mechanisms to overcome the cytotoxic effects of the therapeutic agents (Lippert et al., 2011). In contrast, intrinsic resistance is already established by the time of diagnosis, prior to the initiation of treatment (Diego et al., 2018). Investigations into acquired resistance models have revealed that repeated treatment with chemotherapeutic agents can prompt stable alterations within cancer cells, culminating in diminished drug accumulation, enhanced drug expulsion, modified drug metabolism, and improved DNA repair capabilities (Tyner et al., 2022) (Lackner et al., 2012).

Elucidating the distinct resistance mechanisms for each patient is pivotal for the formulation of efficacious therapeutic strategies in HGSOC (Sundar et al., 2016) (Ling et al., 2005). The inherent genetic variability and diversity within HGSOC can engender the formation of cancer cell subsets that display resistance (Kim et al., 2018). Furthermore, the tumor microenvironment, encompassing the presence of ascitic fluid, can contribute to chemotherapeutic resistance by altering the survival and proliferation of cancer cells (Kim et al., 2016). Many pivotal mechanisms have been elucidated that promote the development of acquired resistance in HGSOC (Wieser & Marth, 2019). These include alterations in drug pharmacokinetics, aberrant target expression and impairment of DDR mechanisms (Vasey, 2003). The elevated expression of drug efflux systems, can facilitate the extrusion of chemotherapeutic agents from cancer cells, thereby compromising their pharmacological efficacy (Wu et al., 2011). Furthermore, alterations in the expression of cellular proteins that participate in the detoxification of drugs, including glutathione S-transferases, can impart resistance to a wide range of chemotherapeutic agents (Turella et al., 2006).

1.4.1 Drug Efflux Transport in HGSOC

The principal drug efflux and membrane transport proteins associated with resistance belong to the ATP-binding cassette (ABC) protein family (Smyth et al., 1998). These are responsible for conferring the MDR phenotype in some cancer types, including HGSOC (Wu et al., 2011 and Gottesman et al., 2002). ABC proteins utilize adenosine triphosphate (ATP) as an energy source to facilitate the movement of a variety of molecules across the cellular membrane (Choi, 2005). Three members of the ABC family have been implicated in mediating chemoresistance in HGSOC (Begicevic et al., 2017). ABCB1 has emerged as a widely investigated and well-documented member of this family (Zappe et al., 2020). It acts unidirectionally to diminish intracellular concentrations of platinum agents in HGSOC (Huang et al., 2006). Individuals with elevated ABCB1 expression exhibit poorer overall survival relative to those with lower ABCB1 expression levels (Ween et al., 2015).

The ABCC1 protein facilitates the efflux of drug compounds and the translocation of conjugated organic anions, such as glutathione and glucuronate, through the cell membrane (Velamakanni et al., 2007 and Kathawala et al., 2015). Existing studies have reported that ABCC1 mRNA expression was increased in HGSOC, including samples obtained from patients who had received cisplatin or carboplatin therapy and subsequently experienced disease recurrence (Wada et al., 2006). Additionally, ABCG2 has been identified as a pivotal factor contributing to chemoresistance in HGSOC, owing to its capacity to mediate the efflux of anticancer therapeutics (Leslie et al., 2005). The expression and activity of drug efflux transporter proteins are central factors driving the development of MDR (Lehne et al., 2000). The enhanced expression of these drug efflux transporter proteins diminishes the intracellular concentration of chemotherapeutic agents, granting a survival advantage to the malignant cells (Wu et al., 2011).

1.4.2 Epigenetic Modifications in HGSOC

Epigenetic modifications can also promote the emergence of resistance by suppressing the expression of genes that are essential for maintaining drug susceptibility and enabling apoptosis (Borley & Brown, 2015). Specifically, epigenetic modifications, such as excessive DNA methylation and histone deacetylation, can suppress the expression of

tumor suppressor genes, thereby enabling cancer cells to bypass cell cycle checkpoints (Quagliano et al., 2020). Modulating epigenetic pathways may offer a viable strategy to circumvent chemotherapy resistance in HGSOC. Epigenetic therapies targeting DNA methyltransferases or histone deacetylases have demonstrated potential in reactivating the expression of silenced tumor suppressor genes, thereby enhancing the effectiveness of conventional chemotherapeutic approaches (Kwon et al., 2011 and Xie et al., 2021). Increased DNA repair capabilities, facilitated by the upregulation of genes involved in NER and HR pathways, can also counteract the cytotoxic effects of platinum-based chemotherapies (Reed et al., 2013). Furthermore, the tumor microenvironment (TME), encompassing factors secreted by stromal cells, may contribute to the progression of resistance (McMullen et al., 2020). Additionally, the metastatic potential of cancer cells and the existence of CSCs have been linked to the development of chemotherapy resistance (Findlay et al., 2014). This subpopulation of cancer cells displays heightened self-renewal capacities and enhanced resistance to traditional therapeutic approaches. The presence of resistant cell populations demonstrating CSCs formation, can contribute to the aggressive defense against treatment (Zhang et al., 2018). The accumulating evidence underscores the complex and multifaceted nature of chemotherapy resistance in HGSOC (Wang et al., 2022, Damia et al., 2019 and Ling et al., 2005).

1.4.3 Apoptosis Mechanism in HGSOC

A principal factor contributing to the poor prognosis of ovarian cancer is the malignancy's ability to evade apoptosis. This tightly regulated process is fundamental for preserving tissue homeostasis and impeding the proliferation of abnormal cells. In general, apoptosis comprises two principal mechanisms: the intrinsic or mitochondrial pathway and the extrinsic or death-receptor pathway (Bucur et al., 2008 and Burz et al., 2009). Various cellular stressors, including exposure to harmful radiation, genetic irregularities, oncogenic signaling, and viral infections are capable of effectively activating the intrinsic apoptotic pathway (Kaufmann et al., 2007). These diverse stimuli trigger the pro-apoptotic Bcl-2 family members, thereby initiating the process of apoptosis-mediated cell death. (Adams & Cory, 2007). This activation results in permeabilization of the mitochondrial outer membrane (MOMP), thereby enabling the downstream release of pro-apoptotic mediators such as cytochrome c (Sellers et al, 1999). This ultimately

triggers the activation of a cascade of caspase enzymes (Kroemer et al., 2007). In the intrinsic apoptosis pathway, the Bcl-2 protein family serves as a critical regulator, where the equilibrium between pro-apoptotic and anti-apoptotic members dictates the cellular outcome (Tsujimoto et al., 2000).

The Bcl-2 family can be subdivided into three functional groups: anti-apoptotic proteins, pro-apoptotic multi-domain effectors, and pro-apoptotic BH3-only proteins. The anti-apoptotic subgroup includes Bcl-2, Bcl-xL, MCL-1, (Sellers et al., 1999 and Elmore et al, 2007). Bcl-2-like 2, Bcl-2 homologue-B, and Bcl-2-related protein A1, which function to sustain cell survival by constraining the pro-apoptotic Bcl-2 members (Ding et al., 2009 and Chipuk et al., 2010). The anti-apoptotic Bcl-2 family, serve to preserve cellular survival by selectively driving the pro-apoptotic Bcl-2 proteins (Delft & Huang, 2006). In contrast, the pro-apoptotic multi-domain effector proteins, such as Bax and Bak, are pivotal in directly inducing the MOMP, thus facilitating the ensuing release of apoptogenic factors.

The pro-apoptotic BH3-only proteins within the Bcl-2 family, such as Bim, Bad, Bid, Puma, and Noxa, function as critical regulators that detect cellular distress. These BH3-only proteins have the capacity to either directly activate the pro-apoptotic multi-domain Bcl-2 effector proteins or indirectly initiate the apoptotic process by suppressing the anti-apoptotic Bcl-2 family members (Shibue et al., 2006).

Caspase-9 is the key regulator of the intrinsic apoptosis pathway, acting as the initiating caspase and subsequently inducing the activation of the effector caspases-3 and -7 (Jacks & Weinberg, 2002).

The extrinsic apoptosis pathway, in contrast, is initiated by the binding of death-inducing ligands, such as Fas ligand or tumor necrosis factor (TNF) -related apoptosis-inducing ligand, to their respective death receptors located on the cell surface (Pfeffer et al., 2018). Binding of death ligands to their respective death receptors on the cell surface initiates the assembly of a death-inducing signaling complex (DISC). The DISC complex consists of the Fas-associated death domain (FADD) as an adaptor, along with procaspase-8, procaspase-10 (Lee et al., 2012). During this assembly, the proactive form of caspase-8 stays bound to the DISC, while the active form dissociates to trigger a cascade of caspase

activation, ultimately driving the execution phase of apoptosis (Peter et al., 2001 and Wang et al., 2010).

EOC commonly have disrupted regulation of key apoptosis controllers, for instance, HGSOC often features the deactivation of the p53 tumor suppressor, which is pivotal for triggering intrinsic apoptosis in response to cellular stress. This dysregulation leads to escalated anti-apoptotic Bcl-2 proteins and reduced pro-apoptotic Bcl-2 proteins, preventing MOMP and allowing these cancer cells to evade cell death (Qian et al., 2022). Strategies to target the dysregulated apoptosis pathways, such as the use of BH3 mimetics to inhibit anti-apoptotic Bcl-2 proteins or the activation of pro-apoptotic Bcl-2 family members, are areas of ongoing research and clinical investigation for HGSOC (Fairlie et al., 2021).

The defining characteristic of the extrinsic apoptosis pathway is its ability to be activated without requiring mitochondrial involvement or the participation of Bcl-2 family proteins (Lavrik et al., 2014). When the extrinsic apoptosis pathway is hindered, the intrinsic mitochondrial route can be activated as an alternative means to induce cell death (Yin, 2000). Furthermore, activated caspase 8 can integrate the intrinsic mitochondrial pathway by cleaving Bid protein (Hassan et al., 2014). Activated caspase-8 can potently activated the intrinsic mitochondrial apoptosis program (Yin et al., 2000). This mechanism bridges the extrinsic and intrinsic death pathways, catalyzing the mitochondrial release of apoptogenic signals and triggering a caspase cascade to orchestrate (Gupta et al., 2001)

1.4.4 Epithelial to Mesenchymal Transition Mechanism (EMT) in HGSOC

The transition from an epithelial to a mesenchymal cellular state is a fundamental process in the progression of cancer that empowers epithelial cells to adopt a more migratory and invasive phenotype, thereby enabling metastasis. EMT is a multifaceted process that involves the corruption of cell-cell contacts, the loss of cellular polarity, and the reorganization of the cytoskeletal network (Singh et al., 2010). These alterations culminate in enhanced cellular mobility. In HGSOC, EMT is considered a pivotal process driving the development and advancement of this aggressive form of cancer. Cancer metastasis begins with tumor cell invasion, which is initiated by a reduction in cell-cell adhesion and an increase in cell motility and invasiveness, both of which are driven by

EMT (Chiang et al., 2016). Several TFs including Snail, Twist, ZEB1, and E12/E47, have been linked to the transcriptional suppression of E-cadherin, which is a key feature of EMT (Son et al., 2010). The loss of E-cadherin expression is a pivotal step in the shift from an epithelial to a mesenchymal state, as it compromises intercellular adhesion and facilitates the detachment of cells from the primary tumor (Lamouille et al., 2014).

Concurrently, there is increased expression of mesenchymal-associated proteins, including vimentin, fibronectin, neural cadherin, β1 and β3 integrins, and matrix metalloproteinases, resulting in the adoption of a more migratory and invasive cellular phenotype (Gerashchenko et al., 2019). The increased expression of the vimentin correlates with enhanced cell motility and a more malignant tumor behavior. Conversely, decreased expression of E-cadherin can activate signaling pathways associated with enhanced cell survival and migration, further increasing the metastatic potential of cancer cells (Datta et al., 2021). SNAIL, SLUG, and TWIST are regarded as key drivers of the EMT. They act to diminish the expression of the epithelial cell-surface protein E-cadherin while enhancing the upregulation of mesenchymal marker proteins. EMT process in HGSOC is believed to be orchestrated by the intricate interplay of various mechanisms, including transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α) /nuclear factor-kappa B (NF-Kβ), and Snail-mediated signaling (Vergara et al., 2010). The transforming growth factor-beta signaling axis exerts a pivotal role in orchestrating the EMT program, resulting in the downregulation of epithelial phenotypic markers and the concomitant upregulation of mesenchymal markers (Yu et al., 2015)

Importantly, the apoptosis signaling pathway is frequently disrupted in HGSOC, which may further enhance the metastatic capacity of the disease by facilitating the survival and persistence of tumor cells undergoing the EMT. The attenuation of caspase-dependent apoptotic signaling cascades, resulting from the decreased expression of the cell-cell adhesion protein E-cadherin, can induce pro-survival signaling mechanisms (Tiwari et al., 2012). This suppresses apoptotic signaling and enables the survival of tumor cells that have undergone a shift towards a more mesenchymal phenotype (Gheldof et al., 2013). EMT is a pivotal mechanism that propels the progression and dissemination of HGSOC. Elucidating the intricate molecular mechanisms and signaling models driving EMT in this

aggressive form of cancer is crucial for designing targeted therapeutic interventions and enhancing patient prognosis (Nam et al., 2014).

1.5 New Strategies to Overcome HGSOC Resistance

Recent research has highlighted potential approaches to address this challenge, focusing on targeting the DDR and other compensatory mechanisms that enable cancer cells to evade the cytotoxic effects of PARPi. The critical importance of the HRR pathway in determining the efficacy of PARPi is emphasized (Xie et al., 2020). Consequently, the combination of PARPi with agents targeting the DDR, such as ATR or CHK1 inhibitors, has emerged as a promising strategy to overcome resistance to Olaparib (Fan et al., 2020). Additionally, a combination therapy study has targeted MDR transporters like P-gp, which can actively remove PARPi from cancer cells (Yokoyama et al., 2017). The utilization of these transporters in combination with olaparib has been demonstrated to enhance the cytotoxic effects of olaparib in preclinical investigations. Developing more robust biomarkers to forecast patients' responsiveness and resistance to PARPi is a crucial area of ongoing research (Keung et al., 2019). Identifying suitable biomarkers can inform the selection of the most appropriate PARP inhibitor and therapeutic approach for individual patients, ultimately enhancing their clinical outcomes (Bitler et al., 2017).

1.6 miRNAs and Specific Roles In HGSOC

Extensive academic research has highlighted the pivotal role of microRNAs (miRNAs) in the initiation and progression of diverse cancer types. These small, non-coding RNA molecules regulate gene expression during transcription (Kinose et al., 2014). Notably, miRNAs have been found to be aberrantly expressed in numerous cancers, suggesting their functions can be context dependent. The regulation of these non-coding RNAs by cancer is shaped by the specific genes they target (Li et al., 2010). For instance, a miRNA that targets a tumor suppressor gene may function as an oncogene, while a miRNA that acts as an oncogene in one cancer type may suppress cancer formation in another. To better comprehend this context-dependent functionality, a deeper examination of miRNA biogenesis is warranted (Zhang et al., 2007).

1.6.1 Biogenesis of miRNAs

miRNAs are produced through a multi-step process. First, miRNA genes are transcribed in the nucleus, generating a primary miRNA transcript (pri-miRNA). This pri-miRNA is then cleaved by the Drosha-DGCR8 complex, creating a precursor miRNA (pre-miRNA). The pre-miRNA is transported to the cytoplasm and further processed by the Dicer enzyme, yielding a mature, double-stranded miRNA. Finally, the mature miRNA associates with the RNA-induced silencing complex, which guides the miRNA to its target mRNA, leading to translational repression or mRNA degradation (Carthew et al., 2009 and Tang et al., 2008).

Substantial research has consistently demonstrated that dysregulated miRNA expression occurs across various stages of cancer development and progression. Aberrant miRNA expression patterns may underlie the defining features of cancer, such as uncontrolled cell proliferation, evasion of programmed cell death, and metastatic dissemination (Garzon et al., 2006). For example, the downregulation of tumor suppressor miRNAs like miR-*34a* and let-7 can result in the upregulation of oncogenes, while the overexpression of oncogenic miRNAs including miR-*21* and the miR-*17-92* cluster can also lead to enhanced oncogene expression (Shah et al., 2012). These miRNA-mediated changes in gene regulation ultimately promote tumor growth and survival (Frixa et al., 2015).

miRNA-based therapeutic interventions, including the utilization of miRNA mimics to reestablish the expression of tumor-suppressor miRNAs and the employment of anti-miRNA oligonucleotides to inhibit the functions of oncogenic miRNAs (Saumet et al., 2014). Preclinical studies have yielded promising results for miRNA-based therapeutic interventions, suggesting they may serve as a novel strategy to augment the efficacy of traditional cancer treatments and overcome drug resistance (Taylor et al., 2014). Emerging research has underscored the potential contribution of miRNAs in facilitating resistance to Olaparib in HGSOC (Li et al., 2021 and Zhu et al., 2019). Specifically, upregulated expression of miR-*21* has been documented in Olaparib-resistant HGSOC cell lines. Upregulation of miR-*21* has been found to target and reduce the expression of the tumor suppressor gene PTEN, leading to the activation of the PI3K/Akt signaling pathway, which has been associated with conferring resistance to Olaparip (Bhere et al., 2020). Furthermore, miR-*21* upregulation may contribute to Olaparib resistance by

enhancing the expression of the MDR1 (Pavlikova et al., 2022). Additionally, miR-*181a*, which can target STING and downregulate the expression of BRCA1, a crucial factor in DNA repair processes, has also been linked to Olaparib resistance (Bustas et al., 2023). Diminished BRCA1 expression may compromise the capacity of HGSOC to effectively respond to Olaparib-induced DNA damage, thus augmenting drug resistance (Biegała et al., 2023). Furthermore, the potential involvement of long non-coding RNAs in Olaparib resistance has been explored, with studies indicating that lncRNA MALAT1 may enhance resistance by modulating the expression of BRCA1 (Yadav et al., 2023).

1.7 miR-188-5p Regulation in Diseases

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a crucial role in regulating gene expression. miR-*188-5p* is one such miRNA that has garnered significant attention in recent years due to its involvement in various physiological and pathological processes. miR-*188-5p* has been implicated in the regulation of multiple biological processes, including cell proliferation, differentiation, apoptosis, and migration (Yang et al, 2020). Its regulatory functions are primarily mediated through its ability to bind to the 3' untranslated regions (3' UTR) of target mRNAs, leading to their degradation or translational repression in many cancer types and non-cancerous diseases.

1.7.1 Effect of miR-188-5p in neurodegenerative diseases

Some studies have shown that miR-*188-5p* plays a critical role in neuroprotection and neurogenesis (Rashidi et.al, 2023). Li and her colleagues showed that in oxygen-glucose deprivation (OGD)-induced human neural cell lines (HNC), expression level of *188-5p* was found upregulated and is believed to promote neuronal survival and regeneration due to its target mRNA, PTEN. miR-*188-5p* has been shown to target and downregulate genes involved in apoptosis, thereby enhancing neuronal survival. By upregulation of miR-*188-5p,* cell viability level was suppressed and apoptosis biomarkers such as caspase-8 and caspase-3 was inhibited, suggesting a protective role against apoptosis. The inhibition of PTEN, a known tumor suppressor and negative regulator of the PI3K/AKT pathway, miR-*188-5p* can activate the PI3K/AKT pathway, promoting cell survival and inhibiting apoptosis (Lijing et al, 2020).

Cerebral ischemia/reperfusion (I/R) injury is a complex pathological condition resulting from the restoration of blood flow after a period of ischemia, often leading to neuronal damage and inflammation. Hou and his team, has investigated the on the role of miR-*188-5p* in regulating the pathological network associated with cerebral I/R injury in rat model by using Middle Cerebral Artery Occlusion Reperfusion (MACO/R) and Oxygen Glucose Deprivation Reperfusion (OGD/R) treatments. The study revealed that miR-*188-5p* was significantly upregulated in I/R rats and OGD/R-induced cells and enhanced neuronal viability and reduced apoptosis were observed with miR-*188-5p* silencing. Related with increase in apoptosis level, Bax, an anti-apoptotic effector protein, level is decreased and cleaved caspase-3 level is reduced (Hou et al., 2023).

Alzheimer's Disease (AD) is characterized by progressive cognitive decline, synaptic dysfunction, and neuronal loss. In another study revealed that down-regulation of miR-*188-5p* in the cerebral cortices and hippocampus of AD patients, suggesting its potential role in the disease's pathophysiology. Dendritic spine and synapse loss are hallmark features of AD, contributing to cognitive deficits. In addition to that, overexpression of miR-*188-5p* has been shown to diminish the decrease in dendritic spine density in rat primary hippocampal neuron cultures exposed to amyloid-beta (Aβ). This indicates that miR-*188-5p* may play a protective role in maintaining synaptic integrity in the presence of AD pathology (Lee et al.,2016, Zhang et. al 2014). The protective effects of miR-*188-5p* in AD may be attributed to its role in regulating synaptic proteins and pathways involved in synaptic plasticity. By targeting genes that promote dendritic spine formation and synaptic function, miR-*188-5p* can enhance synaptic resilience against AD-related cases (Lee et al, 2012).

1.7.2 Effect of miR-188-5p in cardiovascular diseases

Besides the effects of *188-5p* on neurodegenerative diseases, studies were identified the role of miR-*188-5p* on cardiovascular diseases. Sustained cardiac hypertrophy is a significant precursor to an abnormal heart function and is recognized as a crucial factor in the treatment of cardiovascular diseases. DSCAM-AS1, a lncRNA, was found significantly upregulated in cardiomyocytes treated with AngII and by silencing led to a reduction in the expression of hypertrophic biomarkers. Researchers also revealed that DSCAM-AS1 has been found associated with miR-*188-5p* and inhibits its function on the

pro-hypertrophy gene G protein-coupled receptor kinase 2 (GRK2). The anti-hypertrophic effects of DSCAM-AS1 silencing were reversed by inhibiting miR-*188-5p* or overexpressing GRK2. The findings suggest that DSCAM-AS1 acts as a positive regulator of cardiac hypertrophy by decoying miR-*188-5p* and augmenting GRK2 expression (Chen et.al, 2020). Another reversal effects of lncRNAs and miRNAs on cardiovascular diseases was determined in Myocardial ischemia-reperfusion injury (MIRI) which is a critical condition characterized by the restoration of blood flow to ischemic heart tissue. Researchers were found that lncGAS5 and SMAD2 expression levels were upregulated in MIRI, and they have a potential role in the injury process. On the other hand, miR-*188-5p* level was found as downregulated and lncGAS5 inhibited the function of miR-*188-5p*. By overexpressing miR-*188-5p,* MIRI is reduced in myocardial tissue, induced apoptosis and inhibited the expression of pro-apoptotic proteins Bax, cleaved caspase-3, and NF-κB while promoting the anti-apoptotic protein Bcl-2. Also, SMAD2, a key protein in the TGF-β signaling pathway, was identified as one of the mRNA targets of miR-*188-5p* and involved in fibrosis, inflammation, and apoptosis. The protective effects of silencing lncGAS5 can be reversed by inhibiting miR-*188-5p*, underscoring the critical role of miR-*188-5p* in this regulatory axis (Xu et al., 2021). In the context of cardiovascular diseases, in myocardial infarction and reperfusion injury, SNHG15(lncRNA9) was found as regulatory element with the miR-*188-5p*/PTEN. By silencing SNHG15, cardiomyocyte apoptosis was reduced with the help of miR-*188-5p* which target PTEN tumor suppressor gene (Chen et al., 2023).

Abdominal aortic aneurysm (AAA) is a serious health condition characterized by the weakening of the aorta, often associated with aging and degenerative changes and miR-*188-5p* play significant roles in the development and progression of AAA which found as significantly upregulated in aneurysmal tissues compared to control aortae. Also, Immunohistochemistry and fluorescence in situ hybridization revealed that miR-188-5p was predominantly expressed in infiltrated macrophages and T cells within the aortic wall. Inhibition of miR-*188-5p* prevented the enlargement of experimental aneurysms, indicating its role in aneurysm expansion. Additionally, 188-5p inhibition attenuated the infiltration of macrophages, T cells, and reduced neovascularization, suggesting that miR-*188-5p* (Huang et al., 2021).

1.7.3 Effects of miR-188-5p in kidney diseases

Contrast-induced acute kidney injury (CI-AKI) is a significant cause of iatrogenic acute kidney injury (AKI). It is particularly prevalent among patients undergoing coronary angiography and other procedures involving contrast agents. Thus, identifying early biomarkers for CI-AKI is critical for improvement in patient outcomes. In a research paper regarding the role of miR-*188-5p* in the early detection and prediction of CI-AKI, elevated levels of miR-*188-5p* were observed in plasma and renal tissues of patients with CI-AKI. By using *188-5p* mimic molecules, oxidative stress and cellular damage in the tissue samples was suppressed and researchers conclude this result as miR-*188-5p* mimics could be explored as a therapeutic strategy to prevent or mitigate CI-AKI (Yu et al. 2023).

Diabetic kidney disease (DKD) is a significant complication of diabetes, characterized by progressive kidney damage often associated with EMT. Triptolide, a compound derived from the traditional Chinese medicine Tripterygium wilfordii, has shown potential for renal protection. However, its mechanism of action and relation with the downregulation of miR-*188-5p* was investigated by x and her team and results were suggested that, by reducing miR-*188-5p* levels, triptolide alleviates EMT and activates the PI3K/AKT pathway, highlighting the therapeutic potential of targeting miR-188-5p in DKD (Xu et al., 2018).

Renal cell carcinoma (RCC) is one of the most common types of kidney cancer. Despite advancements in treatment, the prognosis for advanced RCC remains poor. In a study that focused on miR-*188-5p* and its effect on RCC has showed that *188-5p* is a potential diagnostic marker. Researchers has suggested that reduced miR-*188-5p* expression level was associated with shorter survival in the TCGA dataset and clinical samples. They showed that with upregulating *188-5p* miRNA level in RCC cell lines; growth, colony formation, invasion, and migration levels were suppressed and by targeting Myristoylated alanine rich protein kinase C substrate (MARCKS) gene, a functional gene in membrane trafficking, AKT/mTOR signaling pathway was affected (Shen et al., 2023).

1.7.4 Effects of miR-188-5p in Liver Diseases

Chronic inflammation in the liver triggers the quiescent hepatic stellate cells (HSCs) and one of the main reasons of hepatic fibrosis (HF). Riaz and his team were presented compelling evidence that miR-*188-5p* is significantly upregulated in various models of liver fibrosis, including those induced by carbon tetrachloride (CCl4) and high-fat diet (HFD), as well as in human non-alcoholic fatty liver disease (NAFLD) patients. This upregulation is consistent with previous reports suggesting that miR-*188-5p* is involved in the pathogenesis of liver diseases. Mimicking the miR-*188-5p* resulted in the up-regulation of HSC activation and proliferation by directly targeting the PTEN through the PTEN/AKT pathway (Riaz et al, 2021).

Hepatocellular carcinoma (HCC) is a highly aggressive liver cancer with a propensity for metastasis, significantly impacting patient prognosis. Understanding the regulatory mechanisms involved in HCC metastasis is crucial for developing targeted therapies (Ogunwobi et al, 2019). miRNAs are key players in regulating various cancer-related processes, including metastasis. Down regulated miR*188-5p* was found as significantly related with metastatic behavior of HCC. miR-*188-5p* mimic transfection significantly inhibited the proliferation and migration of metastatic HCC cells (HLF and LM3) by targeting FOXN2 transcription factor (Wu et al, 2023). Functional assays of *188-5p* were demonstrated that overexpression of miR-*188-5p* inhibited HCC cell proliferation and metastasis in vitro and *in vivo*. Fibroblast growth factor 5 (FGF5) was identified as a key target of miR-*188-5p* and restoring FGF5 expression was showed suppressive effects of miR-*188-5p* on HCC cell growth and metastasis (Fang et al, 2015).

1.7.5 Effect of miR-188-5p in Glioma

About a study in glioma, researchers were revealed that miR-*188-5p* is downregulated in glioma tissues compared to adjacent non-cancerous tissues and lower levels of miR-*188-5p* in glioma tissues correlate with a higher rate of distant metastasis and poorer prognosis, suggesting that miR-*188-5p* may serve as a critical biomarker for disease progression and patient outcomes. Functional assays demonstrated that overexpression of miR-*188-5p* significantly reduces the proliferative and migratory abilities of glioma cells. This is consistent with the role of miR-*188-5p* as a potential tumor suppressor. The study also

identifies XRCC5 as a downstream target of miR-*188-5p*. XRCC5, a component of the DNA repair machinery, has been implicated in tumor progression and resistance to therapy (Pardo et al., 2019). The negative regulation of XRCC5 by miR-*188-5p* suggests a mechanism where miR-*188-5p* exerts its anti-metastatic effects by modulating DNA repair processes and cellular responses to DNA damage. Elevated XRCC5 levels in glioma tissues, coupled with its negative regulation by miR-*188-5p*, indicate that XRCC5 may promote glioma progression and metastasis, potentially through its involvement in maintaining genomic stability and facilitating tumor cell survival under stress conditions (Leng et.al, 2021).

1.7.6 Effects of miR-188-5p in Gallbladder Carcinoma (GBC)

In GBC, miR-*188-5p* was found as significantly downregulated. This finding is consistent with the role of miR-*188-5p* as a potential tumor suppressor in various cancers, including nasopharyngeal carcinoma and liver cancer (Li et al., 2019; Zhang et al., 2021). The correlation between low miR-*188-5p* expression and adverse clinical outcomes—such as larger tumor size, lymph node metastasis, and extensive metastasis—highlights its potential as a prognostic biomarker for GBC. Patients with higher miR-*188-5p* expression demonstrated longer overall survival, which further supports its role as a prognostic indicator. The involvement of miR-*188-5p* in GBC tumorigenesis is linked to the regulation of key signaling pathways such as Wnt/β-Catenin Signaling and this pathway is crucial for cell proliferation and differentiation, and its dysregulation is often associated with cancer progression (Nie et al., 2020). The study's findings suggest that miR-*188-5p* may exert its effects by modulating Wnt/β-catenin signaling, potentially affecting GBC cell behavior and tumor growth.

1.7.7 Effects of miR-188-5p in Retina Blastoma

ID4, known for its role as a transcriptional regulator and a suppressor of cell migration and invasion, is found to be downregulated in Retina Blastoma (Rb) tissues. Yang and her colleagues suggested that ID4 might act as a tumor suppressor in Rb, with its loss contributing to increased tumor aggression and metastasis and the overexpression of miR-*188-5p* leads to a significant downregulation of ID4 by directly targeting its 3′-UTR. This observation aligns with the general role of miRNAs in post-transcriptional regulation of

gene expression. The upregulation of miR-*188-5p* in Rb tissues and its impact on ID4 underscores a potentially critical regulatory mechanism in this cancer type. This study also demonstrates that the miR-*188-5p*/ID4 axis regulates Rb metastasis through the promotion of EMT. Like GBC, miR-*188-5p* also affects Wnt/β-catenin signaling, a pathway well-known for its role in regulating cell proliferation, differentiation, and migration by using ID4 axis (Yang et al., 2019).

1.7.8 Effects of miR-188-5p in Colorectal and Colon Cancer

In the concept of Colorectal Cancer (CRC) progression and miRNAs as a critical regulator, Levels of miR-*188-5p* were found as upregulated in CRC tissues, as well as in various CRC cell lines depend on Wu and her team's research. This overexpression of *miR-188-5p* in CRC cell lines, was characterized as enhanced cell proliferation, migration, and invasion. Also in this study, it was confirmed that Forkhead Box L1 (FOXL1) transcription factor played a role in the crosstalk between miR-188 regulation and downstream Wnt/β-catenin signaling activation (Wu et al. 2022).

Chemotherapy resistance to Oxaliplatin (OXA), a commonly used chemotherapeutic agent for colon cancer, is a significant hurdle in the treatment of colon cancer, often leading to treatment failure and poor patient outcomes (Van et al., 2018). Expression levels of miR-*188-5p* were found as upregulated in OXA resistant SW480 colorectal cancer cell line and the mRNA target of miR-*188-5p* was identified as RASA1. Researchers revealed that downregulation of miR-*188-5p* or overexpression of RASA1 in resistant SW480 cells enhanced their sensitivity to OXA, as demonstrated by increased apoptosis and G1/S cell cycle arrest. This study was highlighted the role of miR-*188-5p* in modulating chemosensitivity in colon cancer cells (Zhu et al., 2021).

1.7.9 Effects of miR-188-5p in Pancreatic Cancer

Pancreatic cancer (PC) is among the most lethal malignancies with a dismal prognosis. Despite advancements in treatment, the survival rate remains low (Bazeed et al, 2022). Zhi and her team revealed that expression levels of LINC00491 in PC tissues and cell lines were enhanced and found related with poor prognosis. The working mechanism behind this phenomenon was characterized with its interaction with miR-*188-5p* and

ZFP91 as a target gene of miR-*188-5p*. In PC cells and mouse tumors, LINC00491 downregulation was associated with increased miR-*188-5p* and decreased ZFP91 levels. Researchers suggested that targeting LINC00491 may offer a novel therapeutic approach for treating PC (Fanget et al, 2022).

1.7.10 Effects of miR-188-5p in Breast and Lung Cancer

Breast cancer (BC) is the most frequently diagnosed malignancy and a leading cause of cancer-related mortality in women globally. Despite advances in treatment, the prognosis for advanced BC remains poor (Lukasiewicz et al, 2021). The Role of *188-5p* in BC was investigated in many studies. In one of the studies, qRT-PCR results were confirmed that 188-5p level was downregulated in BC tissues and cell lines and overexpression of miR-*188-5p* inhibited BC cell proliferation, colony formation, invasion, and migration while inducing apoptosis. miR-*188-5p* mimics was also reduced NF-κB p65(Rel) expression. Researchers were confirmed that miR-*188-5p* act as a tumor suppressor gene in BC (Yang et al, 2020). Also, in another study *188-5p* was identified as tumor suppressor miRNA by targeting Rap2c which is a key regulator of the MAPK signaling pathway. Researchers were concluded that, overexpression of miR-*188-5p* inhibited Rap2c expression, thereby suppressed BC cell proliferation and migration and enhanced apoptosis (Zhu et al, 2020).

Chemoresistance, particularly to doxorubicin, poses a significant challenge in the treatment of BC. In a study about Honokiol, derived from Magnolia plants, was explored honokiol's anti-cancer effect on overcoming doxorubicin resistance in BC, focusing on the role of miR-*188-5p* and the FBXW7/c-Myc signaling pathway. Results were showed that, Honokiol sensitized doxorubicin-resistant BC cells to doxorubicin-induced apoptosis and upregulation of miR-*188-5p* was linked to doxorubicin resistance, and honokiol reduced miR-*188-5p* levels, enhancing doxorubicin sensitivity. In this study, FBXW7 was identified as miR-*188-5p* target and downregulation of miR-*188-5p* by honokiol upregulated FBXW7 and inhibited c-Myc, contributing to increased chemosensitivity (Yi et al, 2021).

In research about the effects of *188-5p* and lung cancer, Transfection with the miR-*188-5p* mimic in *in vitro* lung cancer cells was found significantly reduced cell migration, invasion, and clonogenicity. miR-*188-5p* is negatively regulated the MGAT3 by binding

to its 3' untranslated region (3'UTR). Additionally, overexpression of miR-*188-5p* led to increased E-cadherin levels and decreased expression of Snail and α-SMA, indicating a suppression of the EMT process (Niu et al, 2021).

1.7.11 Effects of miR-188-5p in Gastric and Prostate Cancer

Gastric cancer (GC) is a major cause of cancer-related mortality worldwide. The identification of novel molecular targets is essential for improving GC prognosis and therapy. miRNAs have emerged as critical regulators of gene expression in various cancers (Wu et al, 2020). The role of miR-*188-5p* in GC was investigated by Peng and her team and was found significantly downregulated in GC tissues. Researcher identified that low miR-*188-5p* expression is associated with lymph node metastasis, also overexpression of miR-*188-5p* is significantly inhibited GC cell proliferation, migration, and invasion, and promoted apoptosis by its target, ZF91 function. Study has demonstrated that miR-*188-5p* acts as a tumor suppressor in GC by targeting ZFP91 (Peng et al, 2018). Conversely, in another study miR-*188-5p* level was found significantly upregulated in GC cells and tissues. Gain-of-function analysis were revealed that miR-*188-5p* promoted GC cell proliferation and migration (Wang et al, 2019).

Prostate Cancer (PCa) has high metastatic capacity and elucidation of the molecular targets and pathways regulated by tumor-suppressive miRNAs can provide insights into the oncogenic and metastatic processes in PCa (Kojima et al, 2012). miR-*188-5p* was found as significantly downregulated in metastatic PCa and its low expression was evaluated as an independent prognostic factor. Researchers showed that upregulation of *188-5p* sensitized the PCa cells and inhibited metastasis by suppressing LAPTM4B gene. LAPTM4B can enhanced the multidrug resistance by promoting drug efflux and anti-apoptosis by activating PI3K/AKT signaling. Collectively, these results were concluded that miR-*188-5p* suppressed PI3K/AKT signaling pathway by targeting LAPTM4B (Zhang et al 2015, Lie et al, 2010).

1.7.12 Effects of miR-188-5p in Leukemia

Acute myeloid leukemia (AML) is a prevalent hematopoietic malignancy, and drug resistance significantly impairs the efficacy of chemotherapy (Niu et al, 2022). In a study

about exploring the molecular mechanisms of miR-*188-5p* on doxorubicin resistance in AML, miR-*188-5p* level was found upregulated in AML cells. By inhibiting miR-*188-5p*, doxorubicin resistant AML cells were sensitized to treatment. As a mRNA target of *188-5p*, decreased PTEN expression and subsequent activation of the PI3K/AKT signaling pathway was found as associated with increased resistance to doxorubicin in the presence of miR-*188-5p* inhibitors (Lu et al, 2023).

1.7.13 Effects of miR-188-5p in Ovarian Cancer

miR-*188-5p* have been shown to play critical roles in ovarian cancer (OC) progression. In a study which investigated the role of miR-*188-5p* in OC cells revealed that upregulation of miR-*188-5p* led to significant reductions in migration and increased apoptosis in OC cells. miR-*188-5p* is also determined as target of cyclin D2 (CCND2), an important gene in cell division. miR-*188-5p* function as a tumor suppressor by binding to CCND2, disturbed cell division and eventually increased the apoptosis rate of ovarian cancer (Zhang et al, 2023). miR*188-5p* and its mRNA target FOXN2 were found as downstream targets of a circular RNA (circRNA) named hsa_circ_0001741. In a research study about OC progression, hsa_circ_0001741 expression was found to be reduced in OC cells and tissues and knockout of FOXN2 or increased level of miR-*188-5p* was reduced the anti-proliferative effects of hsa_circ_0001741 on OC cell proliferation (Wang et al, 2024). In the literature, there was only one research paper about HGSOC and miR*188-5p* relation. Depend on bioinformatic analyses, researchers were showed that upregulated lncRNA CTBP1-DT is associated with low survival rate in HGSOC by binding miR*188-5p*. MAP3K, the mRNA target of miR-*188-5p*, was found upregulated in HGSOC and have important function on NF-KB over-activation (Zhang et al, 2019). It was showed that CTBP1 and MAP3K3 bind to *188-5p* from the same seed. Due to the impact of CTBP1 on *188-5p*, *188-5p* could not target the MAP3K, thereby MAP3K3 was protected from degradation (Liu et al, 2021).

1.8 Aim of the Study

The relapse rate of epithelial ovarian cancer patients in the first 3 years after cytoreductive surgery is 70%, unfortunately. The optimal treatment method for platinum-resistant or refractory patients is still not fully known. FDA-approved targeted drugs, such as anti-angiogenic agents like bevacizumab and PARP inhibitors, have not been able to significantly increase survival due to drug resistance. Considering this information, first of the aims of this study is to gain more detailed information about the resistance mechanisms frequently encountered in ovarian cancer treatment. Although the roles of some cell signaling pathways in creating drug resistance have been demonstrated in the literature, more comprehensive and detailed information is needed in this regard. miRNAs, which are non-coding RNA molecules, play an important role in various cellular pathways by suppressing or promoting one or more genes. More than 50% of miRNA genes are in regions of the genome associated with cancer. Many studies in the literature show the significant roles of miRNAs in cancer formation and progression. Within the scope of this study, second aim is to determine the role of miRNAs in the context of treatment response and resistance in HGSOC is explored and synergistic effects of miRNAs with monotherapy or combination therapy strategies with drugs used in the clinic will be tested. This information will shed light on the alternative treatment of HGSOC. Also, in this project, we aim to mimic the acquired resistance in patients in clinic with *ex vivo* primary patient cells. Thus, any result obtained from this study regarding acquired resistance and possible ways of making cancer cells sensitive to drug will have high impact on clinical setting as well.

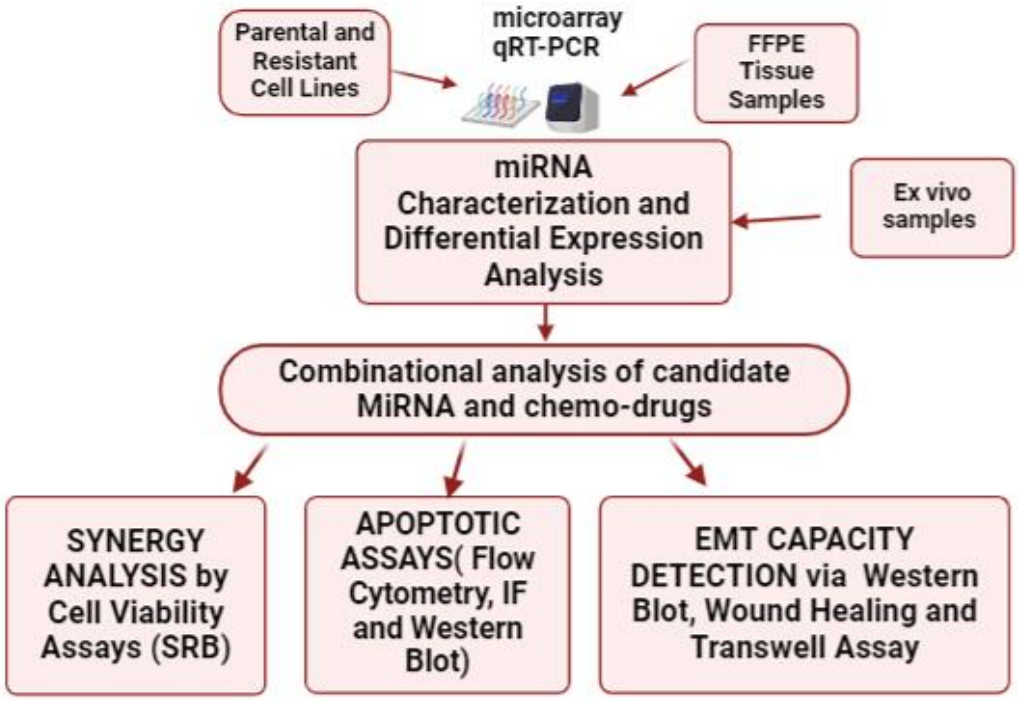


Figure 1.5: Summary of experimental flow in this thesis study (created with BioRender.com)

Chapter 2:
MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipments and materials

Table 2.1: Equipment and materials used in this project.

Pipettes	Eppendorf Reference, Research
Pipette Tips	Sarstedt
Serological Pipettes	Sarstedt
Microfuge Tubes	Eppendorf Safe-Lock Microcentrifuge Tubes
E-plates	Agilent
Cell Culture Plates	Sarstedt
Cell Culture Dishes	Sarstedt
Cell strainers	Sarstedt
Scalpel Handle and Forceps	Electron Microscopy Sciences (EMS)
Microscope Slides	Thermo Fischer
Transwell Inserts (8µM pore size)	Sarstedt
Confocal Microscope	Leica DMi8 /TCS SP8-DLS
Waterbath	Memmert
Centrifuge	Themo Scientific
Inverted Microscope	Nicon Eclipse TS100
Biosafety Cabinet	LABCONCO Class II, Type A2
CO_2 Incubator	New Brunswick Galaxy 170 S
Vortex	Stuart SA8
Heating Block	Denville Scientific Inc. D1100
pH Meter	Mettler Toledo
Microplate Reader	Synergy H1 Plate Reader
Scanner	HP Scanjet G4050

Flow Cytometry	Beckman Coulter CytoFLEX
Cell Analyzer	Guava® Muse® Cell Analyzer
RTCA	Agilent xCELLigence Real-Time Cell Analysis
Transfer System	Trans-Blot® Turbo™
Electrophoresis System	Mini-PROTEAN Vertical Electrophoresis
Western Imaging System	Licor Odyssey® Fc

2.1.2 Chemicals

Table 2.2: *Chemicals, supplier company and catalog numbers used in this thesis.*

Chemical/Solution	Supplier Company/Catalog Number
Dimethyl Sulfoxide (DMSO)	Sigma, #D8418
1X Dulbecco's Phosphate Buffer Saline (DPBS)	Biowest, #L0615
RPMI 1640	Capricorn #RPMI-A
Fetal Bovine Serum (FBS)	Biowest, S1600H
Penicillin-Streptomycin Solution 100X (P/S)	Biowest, #L0022
Amphotericin B	Gibco, #15290018
0.25% Trypsin-EDTA	Multicell
Collagenase/Dispase	Roche, 10269638001
Sulforhodamine B Sodium Salt	Sigma, S1402
Trichlorocetic acid (TCA)	Sigma, T4885
Acetic Acid (Glacial)	Isolab, 901.013.2500
Tris	Biorad, 161-0719
Olaparib (AZD2281)	Selleckchem, S1060
Carboplatin (Clinical Grade)	Kocak Farma, 8680150010030
Crystal Violet	Pro Lab Diagnostics
Methanol	Isolab, 947.046.2500
Ethanol	Isolab, 920.026.2500
Propidium iodide (PI)	Sigma, P4864
TritonX-100	Sigma, X100

RNAse A	Thermo Fischer, 1209021
Paraformaldehyde	Sigma, #158127
Tween-20	Sigma, #P1379
Superblock	ScyTek Laboratories
Mounting Medium with DAPI	Abcam, #ab104139
DAPI and Hoechst Nucleic Acid Stains	Thermo Scientific™, # 62249
RIPA Lysis Buffer	Ecotech ClearBand, RIPA-100
PhosSTOP	Roche, #4906837001
Protease Inhibitor Cocktail	Roche, #05892791001
4x Laemmli Sample Buffer	Biorad, #1610747
2-Mercaptoethanol	Biorad, #1610710
10x Tris/Glycine/SDS Buffer	Biorad, #161077EDU
Blotting-Grade Blocker	Biorad, #1706404
Bovine Serum Albumin	Sigma, #A2153
Sodium Azide	Sigma, #71289
Ponceau S	Ecotech ClearBand, #PS05
Pierce™ ECL Western Blotting Substrate	Thermo Fisher, #32106
Lipofectamine ™ 3000	Thermo Fischer, #L3000001
SYBR Green qPCR Mastermix	Qiagen, #330500
hsa-miR*188-5p* Mimic	Thermo Fischer, mirVana® miRNA mimic, #4464067
hsa-miR*188-p* Inhibitor	Thermo Fischer, mirVana® miRNA inhibitor, #4464084
hsa- miR *let7b-5p* Mimic	Thermo Fischer, mirVana® miRNA mimic, #4464066
mirVana™ miRNA Mimic, Negative Control	Thermo Fischer, 4464058

2.1.3 Commercial Kits

Table 2.3: *Commercial Kits used in this thesis.*

Commercial Kits	Supplier Company/Catalog Number
NucleoSpin miRNA, Mini kit for miRNA and RNA purification	Macherey- Negel, # 740971.50
Total RNA Isolation Kit	Zymo Research, #R1055
RevertAid First Strand cDNA Synthesis Kit	Thermo Fischer, #K1621
Muse® Annexin V & Dead Cell Assay Kit	Millipore, #MCH100105
Muse® Caspase-3/7 Assay Kit	Millipore, #MCH100108
Annexin V-FITC Early Apoptosis Detection Kit	Cell Signalling, #6592
PierceTM BCA Protein Assay Kit	Thermo Fischer, #23225

2.1.4 Antibodies

Tablo 2.3: *Primary antibodies used in this thesis.*

Primary Antibodies	Company/Catalog Number/Dilution
Akt Rabbit mAb	Cell Signaling, #9272S 1:1000
P-Akt (S473) Rabbit mAb	Cell Signaling, #9271L 1:500
PARP (46D11) Rabbit mAb	Cell Signaling, #9532S 1:1000
H2A.X Rabbit Ab	Cell Signaling, #2595 1:1000
Phospho-Histone-H2A.X (Ser139) (D7T2V) Mouse mAb	Cell Signaling, #80312S 1:500
Caspase 8	Cell Signaling, 1:1000
E-cadherin Mouse mAb	Cell Signaling, #14472S 1:1000
N-cadherin Mouse mAB	Cell Signaling, 1:1000
SNAIL Rabbit mAb	Cell Signaling, 1:1000
Alpha Tubulin Rabbit mAb	Cell Signaling, 1:1000

Beta Actin Rabbit mAb	Cell Signaling, 1:1000
Vinculin Mouse Ab	Abcam 1:1000
GAPDH Mouse Ab	Abcam, #ab8245 1:1000

Table 2.4: *Secondary antibodies used in this thesis.*

Secondary Antibodies	Company/Catalog Number/ Dilution
Goat Anti-Mouse IgG H&L (HRP)	Abcam, #ab9723 1:2500
Goat Anti-Rabbit IgG H&L (HRP)	Abcam, #ab6721 1:2500
Alexa Fluor^{TM}594 Goat anti-Mouse IgG	Invitrogen, #R37121 1:10

Table 2.5: *Solutions and preperations used in this thesis*

50%TCA Solution (SRB)	Diluted from 100% Trichloroacetic Acid solution to 50% TCA in ice-cold dH2O
1% Acetic Acid Solution (SRB)	1% (v/v) acetic acid diluted in ddH2O.
SRB Solution	0.4% (w/v) SRB stain dissolved in 1% Acetic acid solution.
10 mM Tris base solution (SRB)	0.3 g Tris base prepared in 500 mL ddH2O.
Propidium Iodide (PI) (Flow)	Dissolved 50 ug PI/ml, 0.1 mg/ml RNase A, and 0.05% Triton100-X in ice-cold 1x DPBS.
Permeabilization Solution (IF)	0.1% TritonX-100 in 100 ml ddH2O
Wash Solution (IF)	0.1% Tween-20 in 100 ml ddH2O

RIPA Lysis Solution (WB)	1X Protease Inhibitor Cocktail and 1X PhosSTOP mixed in 100 ul RIPA Buffer
1x Running Buffer (WB)	100 ml 10x Tris/Glycine/SDS + 900 ml ddH2O
10x TBS (pH:7.6-7.8) (WB)	24 g Tris base + 88 g NaCI + 1 L ddH2O
1x TBS-T (WB)	1 ml Tween-20 + 100 ml 10x TBS + 900 ml ddH2O
Transfer Buffer (WB)	100 ml 10x TGS + 200 ml Methanol + 700 ml ddH2O
Blocking Buffer (WB)	5 g Blotting Grade Blocker + 100 ml 1x TBS-T
10% Sodium Azide ((NaN_3) Solution (WB)	5 g NaN_3 dissolved in 50 ml ddH2O
Primary Antibody Solution (WB)	3% Bovine Serum Albumin (BSA) + 0.02% NaN3 in 1X TBS-T solution
Secondary Antibody Solution (WB)	3% Bovine Serum Albumin (BSA) in 1X TBS-T solution
Primary Antibody Solution (IF)	Diluted in SuperBlock Solution
Secondary Antibody Solution (WB)	Diluted in IF Wash Solution

2.2 Methods

2.2.1 In vitro Cell lines

The study examined three human HGSOC cell lines: OVCAR-3, CAOV-3, Kuramochi and OVSAHO (kind gifts from Professor Ingrid Hedenfalk, Lund University, Sweden). The cells were maintained in RPMI medium supplemented with 10% FBS and 1% P/S, following standard cell culture protocols. Routine testing confirmed the absence of mycoplasma contamination in the cell lines throughout the experiments.

2.2.2 Development of in vitro Resistant Cell Lines

To generate resistant cell lines, the cells were exposed to incremental doses of carboplatin and Olaparib over several months, with the dose being increased as the cells became resistant. The parental cell lines were first plated in cell culture plates. After a 24-hour incubation period, the culture medium was replaced with the starting dose of the respective drugs, which was determined as the IC_{10} concentration. The cells were then maintained in the presence of the drugs, with the culture medium being replaced every 2-3 days. Once the cells reached approximately 70-80% confluency, they were washed twice with 1X phosphate-buffered saline and then sub-cultured in fresh medium containing drug concentrations that were doubled from the previous levels. The maximum tolerated drug concentration that enabled continued cellular growth was identified. Resistance profiles were assessed for both therapeutic agents across the three cell line models. Resistance was developed by incrementally increasing the drug concentrations from 10% of the half-maximal inhibitory concentration (IC_{50}) up to a minimum of the twice-maximal inhibitory concentration (IC_{200}). The resistance development profiles, and resistance indexes of the generated resistant cell lines were assessed through SRB cell viability assays and clonogenic survival assays.

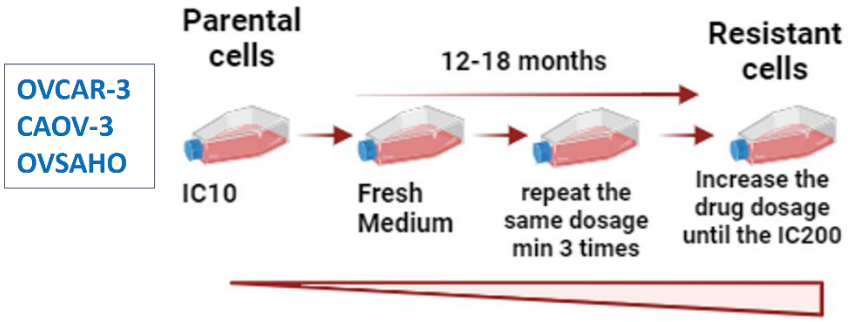


Figure 2.1: Generation of Resistant *in vitro* Cell Lines.

2.2.3 Cell Viability Tests

SRB

Cell viability level of olaparib and carboplatin drugs for resistant and parental cells were evaluated by SRB cell viability test and clonogenic assay. For this purpose, cells were seeded in 96 well plates at a density of 5000 cells/well, treated with 5 different concentrations of olaparib and carboplatin and DMSO (40,20, 10,5, 2.5 uM). At the end of the 5 days incubation, cells were washed with 1xPBS and fixed with 10% Trichloroacetic Acid. SRB dye dissolved in 1% acetic acid was used to stain the fixed cells and excess dye was washed off with 1% acetic acid. The stained cells were solubilized in 10 mM Tris, and absorbance was measured at 570 nm by plate reader. Viability was normalized to DMSO treated control cells. The resistant index calculation of the resistant cells was made by comparing with the parental cell lines.

Clonogenic Assay

For clonogenic assay, experiment and control group cells were seeded at a density of 500 cells/well in 24-well plates. Next day after cells were attached, treatment with olaparib and carboplatin drugs were applied by serial dilution method. When 5 days of treatment is finished, medium was replaced with fresh growth media and refreshed when it is necessary. After 14 days colonies were fixed with 10% formalin, stained with 0.1% crystal violet, and scanned by scanner device. Counting of colonies was performed using Image J software, with Plugin.

2.2.4 Obtaining Patient-Derived Samples

Through a collaborative effort with the gynecologic oncology and pathology department at Koç University Hospital, formalin-fixed paraffin-embedded samples and post-operative tissue specimens were obtained, which were identified as high-grade serous ovarian cancer by Prof. Dr. Çağatay Taşkıran, Dr. Doğan Vatansever, and Dr. Burak Giray.

The patient-derived samples were obtained from two different study groups; Group 1: patients diagnosed with high-grade serous ovarian cancer with good disease prognosis after maximal primary cytoreductive surgery (responding to chemotherapy). Group 2: patients diagnosed with high-grade serous ovarian cancer with poor disease prognosis after maximal primary cytoreductive surgery (resistant to chemotherapy)

Formalin-fixed, paraffin-embedded (FFPE) tissue samples obtained from 40 high grade serous epithelial ovarian cancer patients were studied for miRNA expression profiling. 20 of the FFPE specimens were further utilized to perform differential miRNA expression analysis using microarray assay and 40 of them were used for qPCR experiments. Additionally, *ex vivo* primary cells derived from patient samples were utilized in experiments investigating the combinational effects of miRNA mimic/inhibitors and Olaparib and carboplatin drugs.

2.2.5 Establishment of Ex-Vivo Cell Culture

Tissue samples obtained from patients during surgical procedures were aseptically collected in sterile cold container filled with 1x DPBS and sectioned into small fragments (2 mm x 2 mm) by using sterile forceps and scalpels in the 100mm x100mm glass petri dish. Small fragments of tissues were incubated in 5 ml Collagenase/Dispase (Roche, #10269638001) (3 mg/ml; 2.4U/ml dispase, 0.3 U/ml collagenase) solution for 1 hour at 37ºC to digest extracellular matrix and release single cells. The cells were then passed through a 20 μM cell strainer to remove any undigested tissue debris. The cell suspension was centrifuged at 1500 rpm for 5 minutes. Then, supernatant was discarded, and cell pellets were resuspended in DMEM: F12 medium supplemented with 0.1% Ampicillin B, 1% penicillin/streptomycin, and 20% FBS. The cell suspension was then plated onto

appropriate cell culture dishes under standard tissue culture conditions. Medium was refreshed every 2-3 days. Once the cells had reached 70-80% confluency, they were harvested using trypsin and subcultured into appropriate cell culture plates for further experiments.

2.2.6 Microarray Experiment for Differential Expression of miRNAs

Total RNA was isolated from total 17 formalin-fixed, paraffin-embedded tumor tissue samples using the MN miRNA tissue analysis kit protocol. Isolated RNA samples were then analyzed through Macrogen company using GeneChip™ miRNA 4.0 Array technology. In this experiment, 1 ug total RNA was used for each sample, by using the FlashTag™ Biotin HSR RNA Labeling Kit following by kit manufacture. Biotin-labeled samples were hybridized using GeneChip® Hybridization Oven to the Affymetrix miRNA microarray according to the protocols provided by the manufacturer. Arrays were scanned using the GeneChip® Scanner. During differential miRNA expression analysis, 9 sensitive FFPE samples were used as control group and 8 resistant FFPE samples considered as experimental group. The Affymetrix data extraction protocol and software were utilized to automatically obtain the raw data. The raw microarray data was processed using Affymetrix Power Tools software, which included importing the CEL files, performing miRNA-level RMA+DABG-All analysis, and exporting the results. Moreover, the microarray data was restricted to include only the probe sets designated for the applicable biological subjects. Comparative analysis between the test and control samples was performed by pooling patient data results in two subgroups.

2.2.7 Real Time Quantitative PCR Experiment for Differential Expression of miRNAs

The key 10 miRNAs were identified from the patient-derived samples were subsequently validated through real-time PCR (qRT-PCR) experiments that targeted non-coding RNA transcripts. In this context, isolated total RNAs were converted into cDNA at 500ng/ul concentration using the RevertAid RT Reverse Transciption Kit. LightCycler 480 SYBR Green I Master Kit was used for qRT-PCR analysis. For qRT-PCR studies, stem-loop reverse transcriptase primers and forward primers were designed and synthesised separately for each of the selected miRNAs (Table 2.6). The universal reverse primer was designed and synthesized for all miRNA targets (Table 2.7). As the stem-loop reverse

transcriptase primers were longer than 50 bases, they were purified by high performance liquid chromatography instead of the standard primer purification method. U6 was selected as an endogenous control gene for quantitative real-time PCR (qRT-PCR) studies and forward and reverse primers were designed for this gene (**Table 2.8**).

Table 2.6: *Stemloop RT and Forward Primers of miRNAs*

miRNA	Stemloop Reverse Transcriptase Primers 5'-3'	Forward Primers 5'-3'
miRlet7b-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA ACC AC	TCG CTT GAG GTA GTA GGT TGT
miR19a-3p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT CAG TT	TCG CTT GTG CAA ATC TAT GCA A
miR19b-3p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT CAG TT	TCG CTT GTG CAA ATC CAT GCA A
miR20a-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TAC CT	TCG CTT AAA GTG CTT ATA GTG C
miR106a-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TAC CT	TCG CTA AAA GTG CTT ACA GTG C
miR146a-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA ACC CA	TCG CTT GAG AAC TGA ATT CCA
miR188-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC CCT CC	TCG CTC ATC CCT TGC ATG GT
miR20b-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TAC CT	TCG CTC AAA GTG CTC ATA GTG C
miR4800-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT CCT TC	TCG CTA GTG GAC CGA GGA AG
miR5571-5p	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACG GGA GG	TCG CTC AAT TCT CAA AGG AG

Table 2.7: *Universal Reversal Primer.*

Universal Reversal Primer 3'-5'	GTG CAG GGT CCG AGG T

Table 2.8: *U6 Internal Control Gene Forward and Reverse Primers*

	Forward Primer 5'-3'	Reverse Primer 5'-3'
U6	CTC GCT TCG GCA GCA CA	AAC GCT TCA CGA ATT TGC GT

2.2.8 Investigating the Effects of miRNAs on Cell Proliferation

Lipofectamine Transfection

Based on the verification of miRNA expression levels, 2 key miRNAs were identified through the research findings and literature review, and these were further investigated in the subsequent stages. The mimic products of the identified key miRNAs were procured and introduced to the cell lines. 250.000 cells per well were initially seeded in 6 well plates, and after a 24-hour incubation period, the medium was replaced with an antibiotic-free formulation. Mimic/ inhibitor molecules were dissolved in rnase free water at 100ng/µL concentration and complexed with lipid-based transfection reagent, Lipofectamine, according to the manufacturer's instructions. Transfection mixtures were added to the cells and incubated for 24 hours. When incubation is terminated, medium is replaced with normal growth medium. Cells were harvested at indicated time points and pellets were collected for further analysis.

Table 2.9: *Lipofectamine transfection mixture.*

Transfection Mixture (per well)	
Lipofectamine 3000 Reagent	7.5 µl
Mimic/Inhibitor/Negative Control Molecule	300ng (3 µl)
Antibiotic and FBS free medium	250 µl

Combinational Analysis of the Selected miRNA with conventional Drugs

Transfected resistant cells with selected miRNA mimics were treated with chemotherapeutic agents currently used clinically for ovarian cancer treatment (Olaparib and carboplatin) to perform a synergy analysis by SRB cell viability assay. The following combination and monotherapy treatments were applied to each of the resistant cell groups

as; Olaparib, DMSO negative control (Since Olaparib are dissolved in DMSO), miRNA mimic +DMSO, miRNA mimic +Olaparib. Negative control samples were used as negative control in these experiments. After the specified incubation times, The IC_{50} values of each treatment were calculated by normalizing DMSO control and the synergistic effects of the selected miRNA with the drugs were determined. The results were compared with monotherapy and combination therapy with miRNAs, and the most effective treatment strategy was determined.

2.2.9 Western Blot Experiments

For Western blot analysis, 25 μg of protein samples from parental and resistant cells treated with the combinational and monotherapy (Olaparib or Carboplatin, DMSO negative control, miRNA mimic +DMSO, miRNA mimic +Olaparib) will be run on 4-15% Biorad ready-made gels, and transfer will also be performed using the same system. For this experiment selected miRNA-mimic was used as mentioned at Method 4.7. Membranes will be blocked with blocking solution (%5 milk powder in 1xTBS-T (0.1% tween)) and incubated overnight at +4°C. After incubation with primary antibodies (cleaved-PARP, cleaved caspase-9, cleaved caspase-8…etc) for 1 or 2 hours at room temperature, unbound antibodies washed with 1xTBS-T (0.1% tween) for 30 minutes. Then, the second antibody was applied to the membrane at room temperature for 1 hour. After washing for 30 minutes, the proteins visualized with ECL-Plus. The effects of conventional drugs in combination with miRNA on EMT were investigated using EMT antibodies (E-cadherin, N-cadherin, Snail + Slug, Vimentin, TWIST, Fibronectin) to examine changes in the levels of these proteins.

2.2.10 Determination of the effects of selected miRNAs on Cell Cycle

Parental and transfected resistant cells treated with the combinational and monotherapy (Olaparib or Carboplatin, DMSO negative control, selected miRNA mimic +DMSO, selected miRNA mimic +Olaparib) were collected with trypsin and fixed with cold ethanol at the end of the treatment for analysis by flow cytometry. After the cells have been fixed with 70% ethanol, stained with Propidium Iodide (PI) (marker that binds to DNA) and analyzed by the flow cytometry device.

2.2.11 Annexin V- PI Apoptosis Detection by Flow Cytometry and Immunofluorescence Assays

Parental and transfected resistant cells treated with the combinational and monotherapy (Olaparib or Carboplatin, DMSO negative control, selected miRNA mimic +DMSO, selected miRNA mimic +Olaparib) were collected with trypsin and fixed with cold ethanol at the end of the treatment for analysis by flow cytometry were collected using trypsin and then centrifuged. Next, they resuspended with 1X binding buffer and stained with Annexin V-FITC and propidium iodide (PI). The samples were kept in a dark environment at room temperature for 5 minutes. Finally, they were placed on a slide and covered with a cover slip and examined by fluorescence microscope.

2.2.12 P-h2AX Immunofluorescence Staining

p-h2AX Immunofluorescence staining was performed to assess degree of DNA damage in control and mimic transfected cells. For this assay, cells were seeded in 6 well plates including cover slides, treated with drugs, and fixed with 4% formaldehyde. Fixed cells were permeabilized with 0.1% Triton X-100. Next, slides were blocked with SuperBlock solution and incubated with anti-γh2AX antibody for overnight (1:100 diluted in blocking solution). Next day, slides were washed with IF wash solution and incubated with Alexa Fluor 594-conjugated secondary antibody for 2 hours, at RT. Cover slips were inverted on the clean slides and mixed with 7.5 µl mounting medium containing DAPI to stain nuclei. The slides were imaged using confocal microscope.

2.2.13 Screening Apoptosis Genes by q-RT-PCR experiment

In this context, isolated total RNAs were converted into cDNA at 1000ng concentration using the RevertAid RT Reverse Transciption Kit. LightCycler 480 SYBR Green I Master Kit was used for qRT-PCR analysis. For Apoptoosis Screening qRT-PCR studies, total 80 apoptosis pathway related genes were included and forward and reverse primers were kind gift from Dr. Zelal Adıgüzel, and Dr. Ceyda Açılan Ayhan, Koç University. Master mix preparation for these experiments were given and two qPCR plate templates were followed during these experiments (Figure 2.1). GAPDH is used for internal control gene.

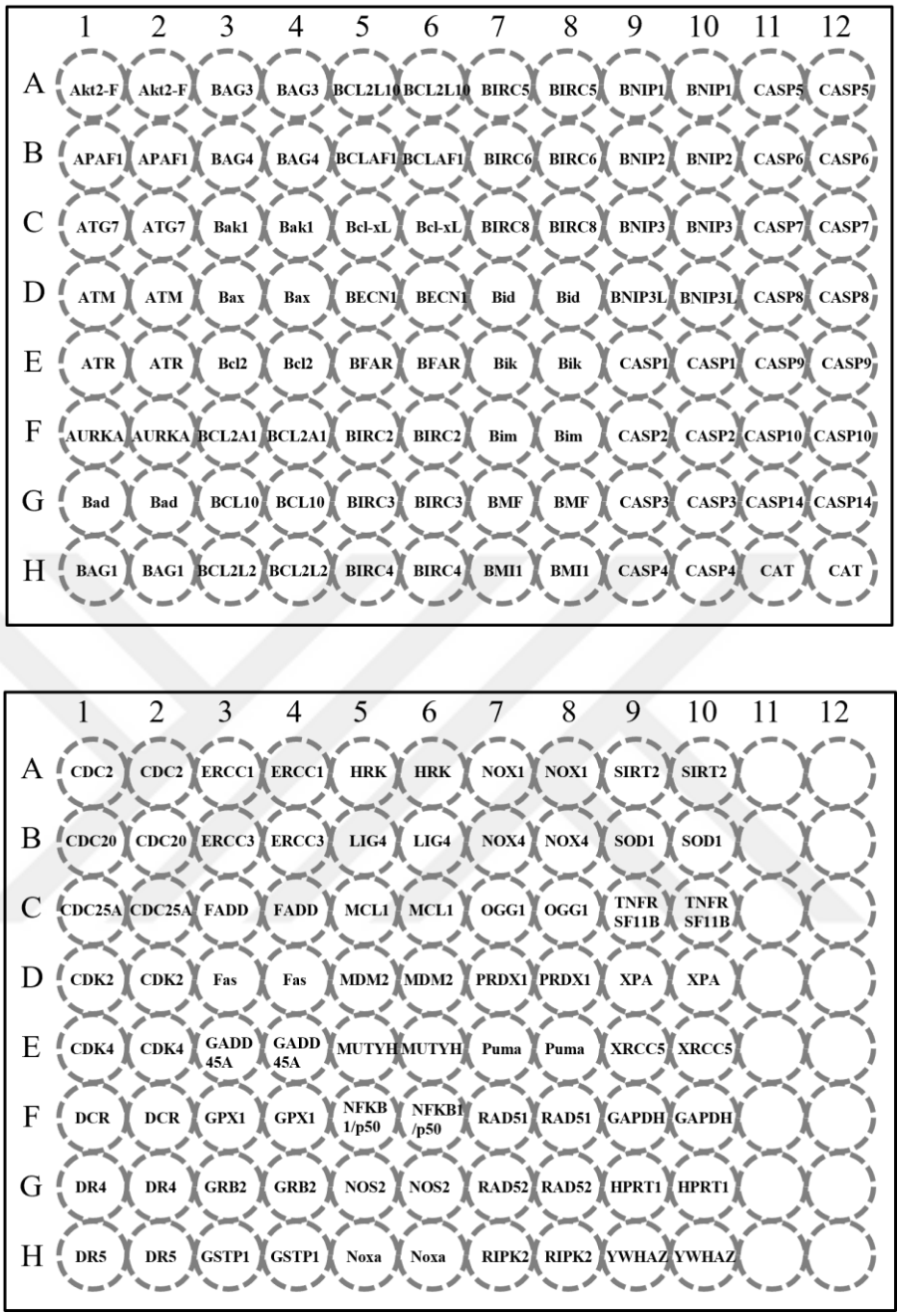


Figure 2.2: *Apoptosis screening plate layout.*

2.2.14 Investigation of the Effects of Combined Application of miRNA with Conventional Drugs on EMT mechanism

Wound Healing Assay

Parental and resistant cells treated with the combinational and monotherapy (Olaparib or Carboplatin, DMSO negative control, selected miRNA mimic +DMSO, selected miRNA mimic +Olaparib) were seeded into 6-well plates and a single vertical wound was created in each well using a 200µl pipette tip when they reach 100% confluency. Then, the cell culture medium was aspirated and replaced with the monotherapies and combinational therapy with mimic. Cell behavior was observed under a microscope for 5 days and wound healing abilities were determined.

Transwell Assay

The migratory capacity of miRNA and NC-transfected cells and control parental cells was assessed using transwell assay. 8 µM pore size- transwell inserts were seated on 24 well plates and 50.000 cells in 100 µL per well were loaded in the upper side of the chamber and allowed to migrate through pores towards the lower part containing no FBS growth medium for 3 and 5 days. The migrated cells were imaged under florescence microscopy after staining with DAPI dye.

2.2.15 Statistical Analysis

When comparing two groups, statistical analysis was performed using the Student's T-test. When examining differences between groups, the multiple t test method or ANOVA was used. Statistical analyses were conducted using the SPSS program. A significance value of $P<0.05$ were used. The applications used for miRNA expression analysis will be determined in collaboration with the contracted company.

Chapter 3:

RESULTS

3.1 Characterization of Resistant In Vitro HGSOC cell lines

3.1.1 SRB Cell Viability and Colony Formation Capacity of Parental and Resistant Cells

Tablo 3.1: Determination of IC_{50} values and resistant index of resistant OVCAR-3, CAOV-3 and OVSAHO cells. Parental cells and resistant cells were studied as two separate groups. Cells were treated with olaparib and carboplatin drugs in eight different concentrations using serial dilution method. After 5 days from incubation, SRB density was measured with an ELISA reader, and the IC_{50} concentrations of the drugs in resistant and parental cell lines were calculated. The drug resistance index was calculated separately for all cell lines by dividing the IC_{50} values of resistant cell lines by the parental control cell lines.

Cell Line	Drug	Parental-IC_{50}	Resistant-IC_{50}	RESISTANT INDEX
OVSAHO	OLAPARIB	2.1 µM± 0.3	9.5 µM± 0.8	4,5
	CARBOPLATIN	1.5 µM± 0.4	8.5 µM± 1	5,7
OVCAR-3	OLAPARIB	0.3 µM± 0.01	2,1 µM± 0.3	7
	CARBOPLATIN	0.3 µM± 0.07	0.6 µM± 0.05	2
CAOV-3	OLAPARIB	2.6 µM± 0.3	6.6 µM± 0.3	2,5
	CARBOPLATIN	4.2 µM± 1	6.3 µM± 0.5	1,5

To determine the effect of differential expression of miRNAs that revert drug resistance in *in vitro* samples, we generated carboplatin and olaparib resistant OVCAR-3, OVSAHO and CAOV-3 cells using stepwise dose increase method. We confirmed the resistant phenotype by SRB cell viability and clonogenic assay. IC_{50} values were calculated via the SRB assay for olaparib- and carboplatin resistant CAOV3, OVCAR3, and OVSAHO cell lines, as well as parental cells as a control. Depend on comparison of parental and resistant cell's IC_{50} concentrations, we determined resistant index values for olaparib resistant OVCAR-3, OVSAHO and CAOV-3 cells as 7, 4.7 and 2.5 and and carboplatin resistant OVCAR-3, OVSAHO and CAOV-3 cells as 2, 5.7, and 1.5 respectively (Tablo 3.1).

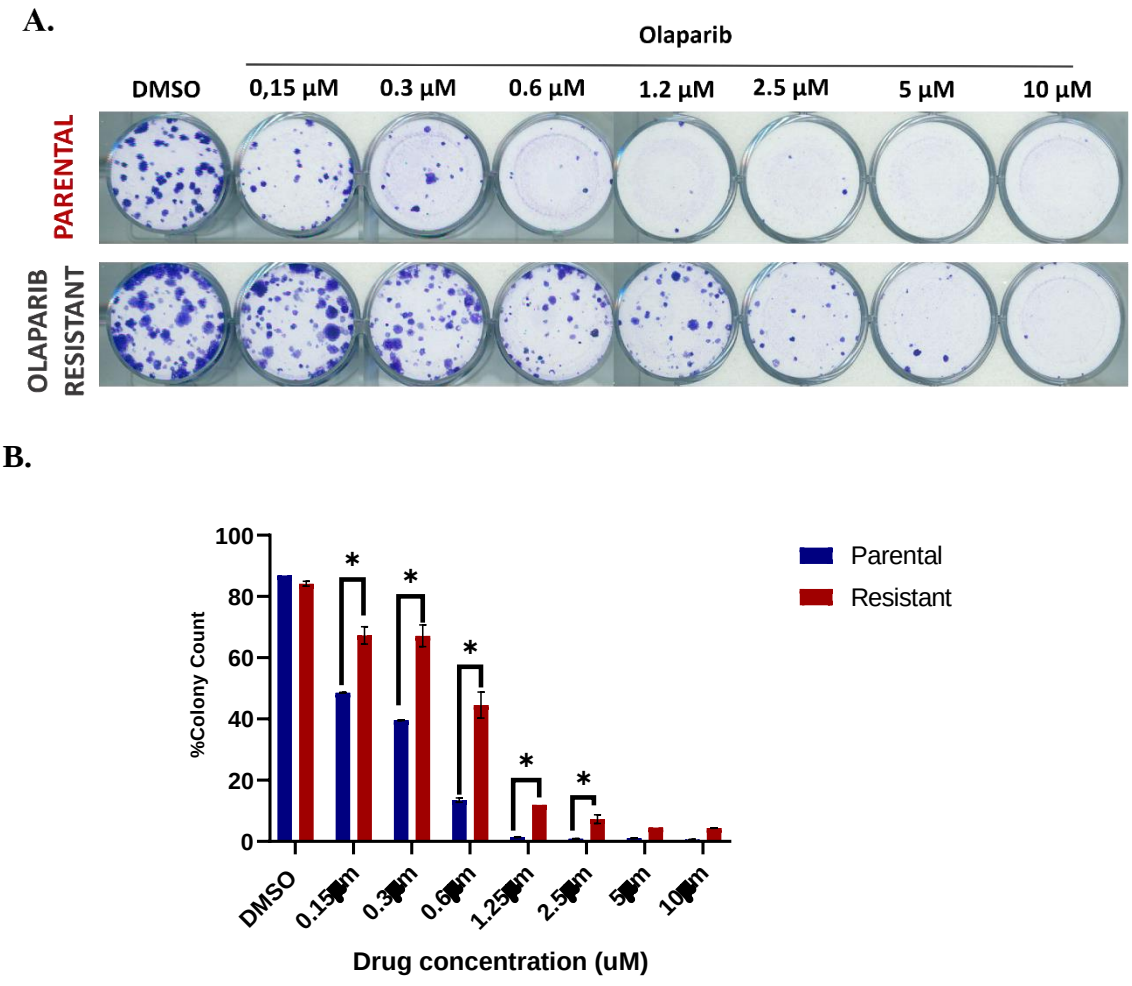


Figure 3.1: Representative Image of Characterization of acquired olaparib resistance in OVCAR-3 cells by clonogenic assay (A). Resistant cells were selected based on a strategy of pulsed exposure to olaparib with time-stepwise increments in dosage. B) Quantification of colonies were assessed by Image J programme. Statistical analysis was made via Student T test, * $p<0.001$.

In addition to SRB test, a colony formation assay was performed to show the development of resistance in cells (Figure 3.1A). Colony formation capacity of the most resistant cell line, Olaparib resistant- OVCAR-3, was tested. Quantification analysis of colonies was showed that resistant cells have more colony formation capacity than parental cells significantly (Figure 3.1B).

3.1.2 Morphological Changes in Resistant Cells

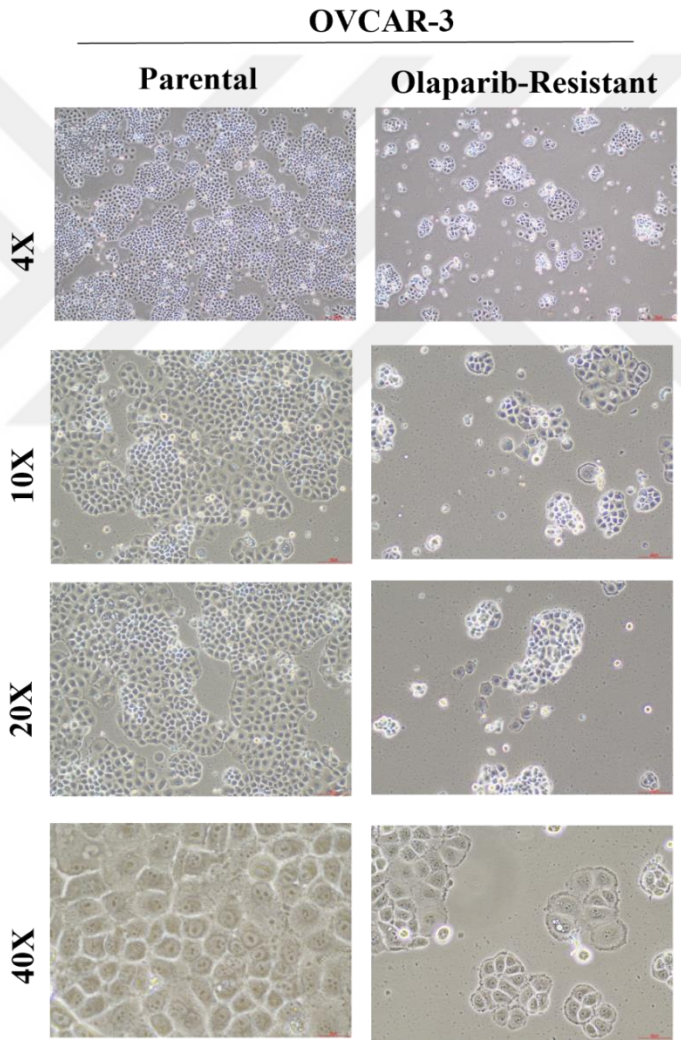


Figure 3.2: The microscopic images of morphological changes of resistant cells that occur along with the development of resistance to olaparib drug in OVCAR-3 resistant cells. The changes were evaluated by comparing them to parental control cells. Images were taken by inverted microscope with 4X,20X and 40X magnification.

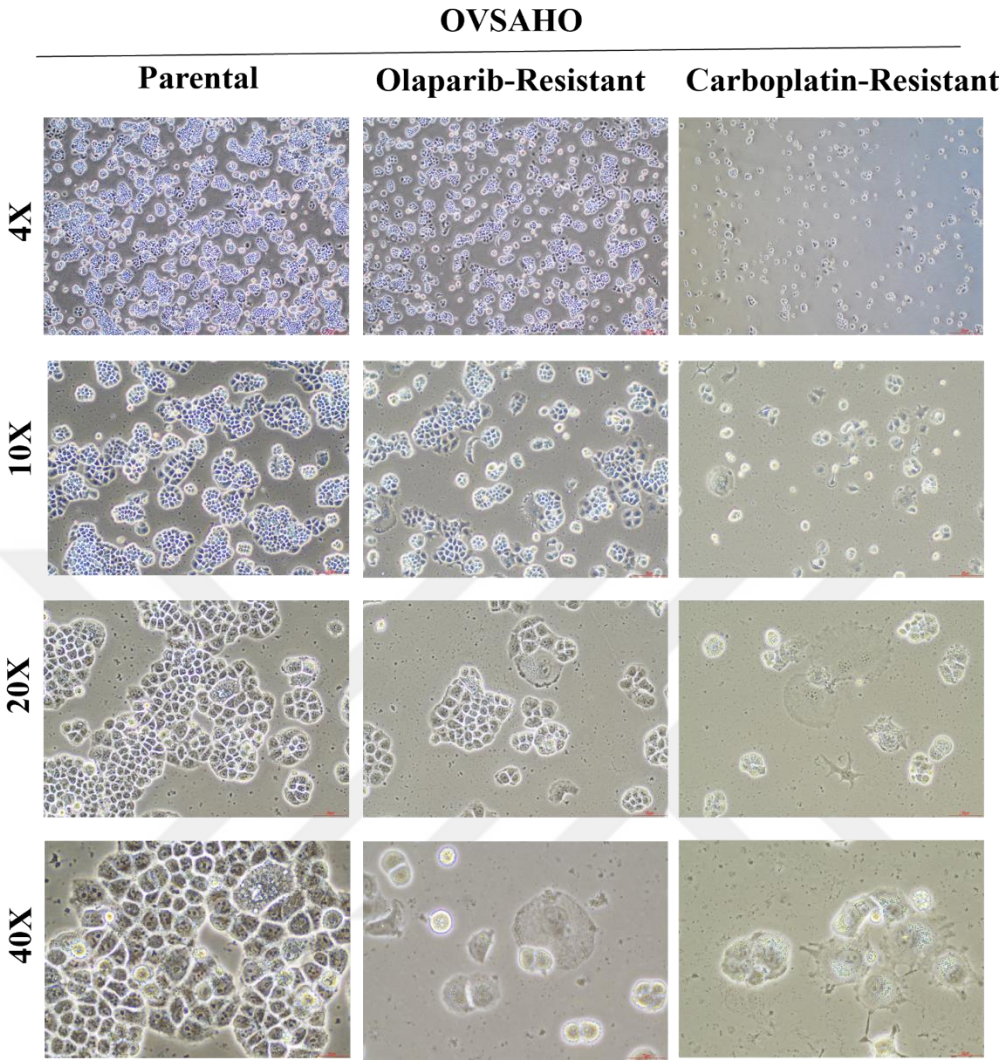


Figure 3.3: The microscopic images of morphological changes of resistant cells that occur along with the development of resistance to olaparib and carboplatin drug in OVSAHO resistant cells. The changes were evaluated by comparing them to parental control cells. Images were taken by inverted microscope with 4X,20X and 40X magnification.

Under microscopy, we have observed the differentiated cell morphologies and stem cell-like properties in OVCAR-3 and OVSAHO resistant cell lines **(Figure 3.2 and 3.3)**. After confirmation of the resistance capacity of cells with cell viability assays and microscopic examination, Resistant cells were treated with IC_{200} concentration of Olaparib drug and after 5 days of treatment their morphologies were evaluated compared with DMSO treated resistant cells. As expected, resistant cells show no changes in their proliferation capacities depend on microscopic observation. Representative microscopic images of D DMSO control and IC_{200} (30µm) Olaparib treated OVCAR-3 cells were shown in **Figure 3.4**.

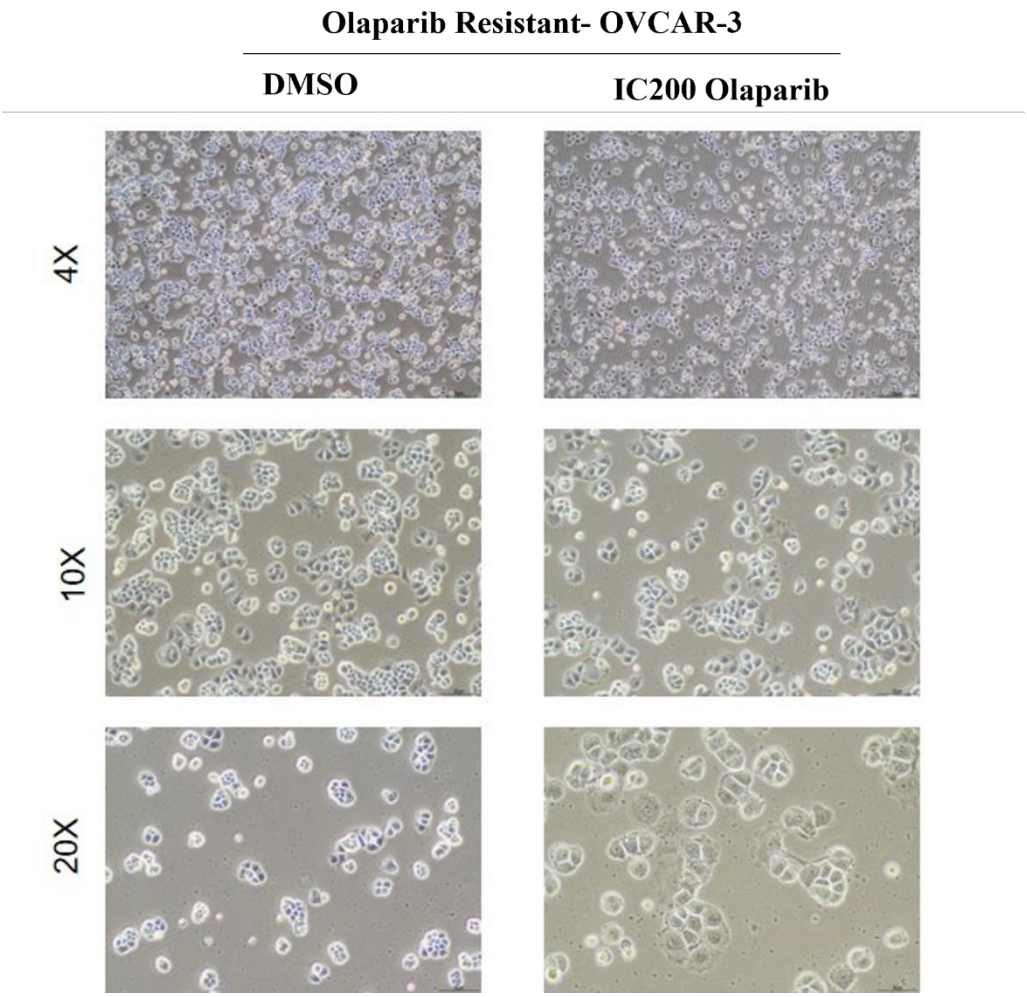
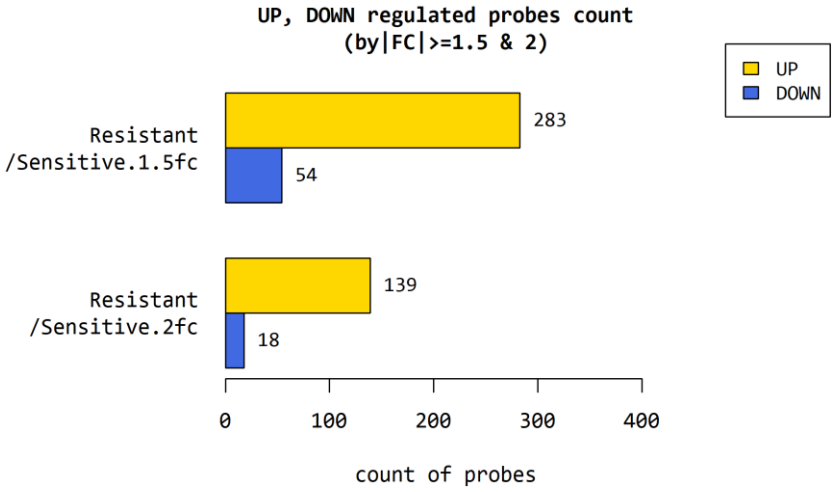


Figure 3.4: The microscopic images of morphological and proliferation changes of OVCAR-3 resistant cells after treatment with IC_{200} concentration of olaparib drug in. The changes were evaluated by comparing them to DMSO treated control cells. Images were taken by inverted microscope with 4X,20X and 40X magnification.

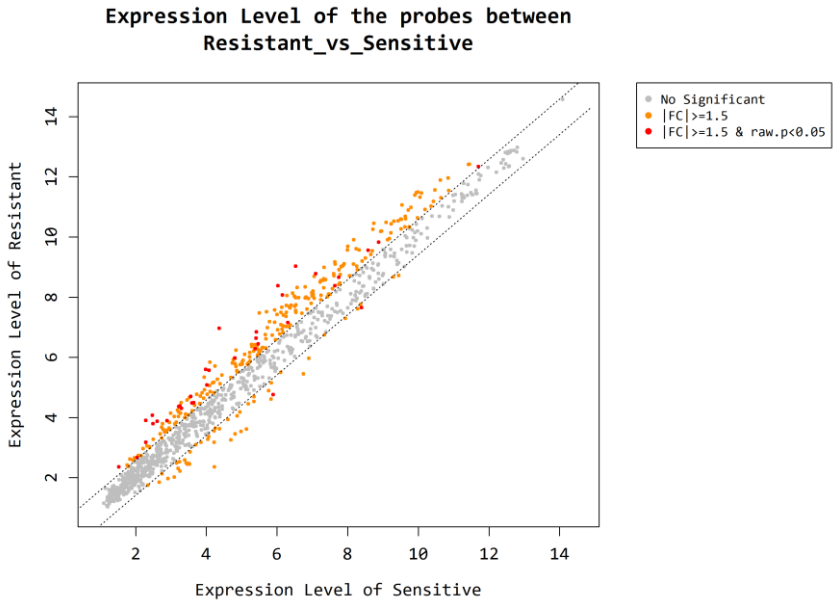
3.2 Differential Expression Analysis of miRNAs in Sensitive and Resistance Samples

Differential expression analysis of miRNAs in HGSOC were performed for sensitive and resistant FFPE tissue samples and *in vitro* parental and resistant OVSAHO, OVCAR-3 and CAOV-3 cell lines by microarray and qRT-PCR experiments. For this purpose, sensitive and resistant FFPE patient samples miRNA expression analysis were performed by microarray experiment and fold change values were determined **(Figure 3.5).**

A.


UP, DOWN regulated probes count
(by|FC|>=1.5 & 2)
UP
DOWN
Resistant
/Sensitive.1.5fc
283
54
Resistant
/Sensitive.2fc
139
18
0
100
200
300
400
count of probes

B.


Expression Level of the probes between
Resistant_vs_Sensitive
No Significant
|FC|>=1.5
|FC|>=1.5 & raw.p<0.05
Expression Level of Resistant
2
4
6
8
10
12
14
Expression Level of Sensitive

C.

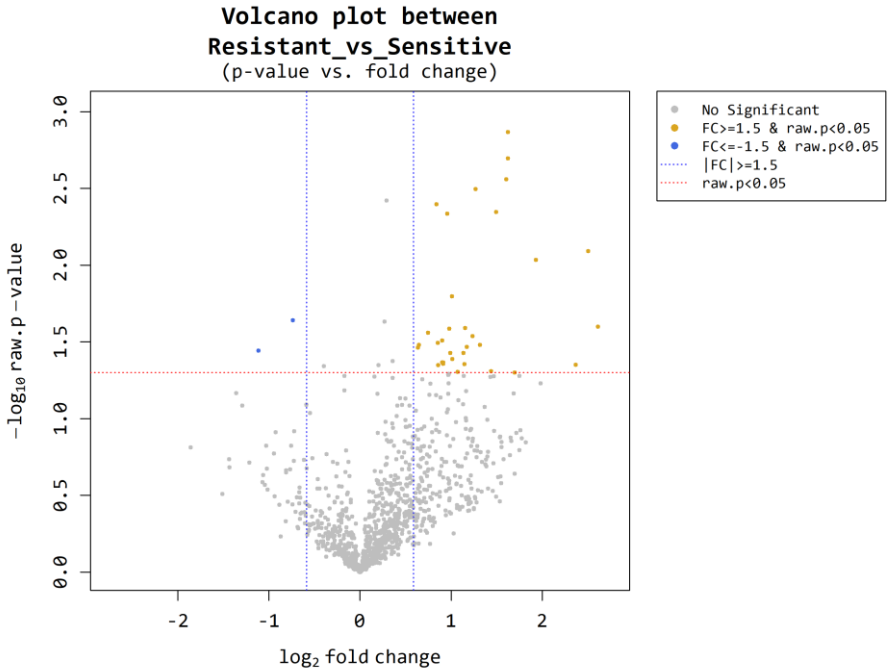


Figure 3.5: Differential miRNA gene expression levels were determined in resistant FFPE samples. Representative images of significant prob counts (A) and expression level of plots (B) volume plots (C) of probes for miRNAs between sensitive and resistant FFPE samples were showed. Fold change value>|1.5| was determined as significant.

To verify the differential expression levels of miRNAs in resistant HGSOC tissue samples by qPCR experiments, 10 candidate miRNAs were determined. Stemloop RT primers, forward primers and universal reverse primers were synthesized for 10 candidate miRNAs and literature search was performed. qRT-PCR assays were performed for 40 FFPE samples (20 Sensitive, 20 Resistant) and parental and resistant *in vitro* cell samples for further investigation of differential analysis **(Figure 3.6)**.

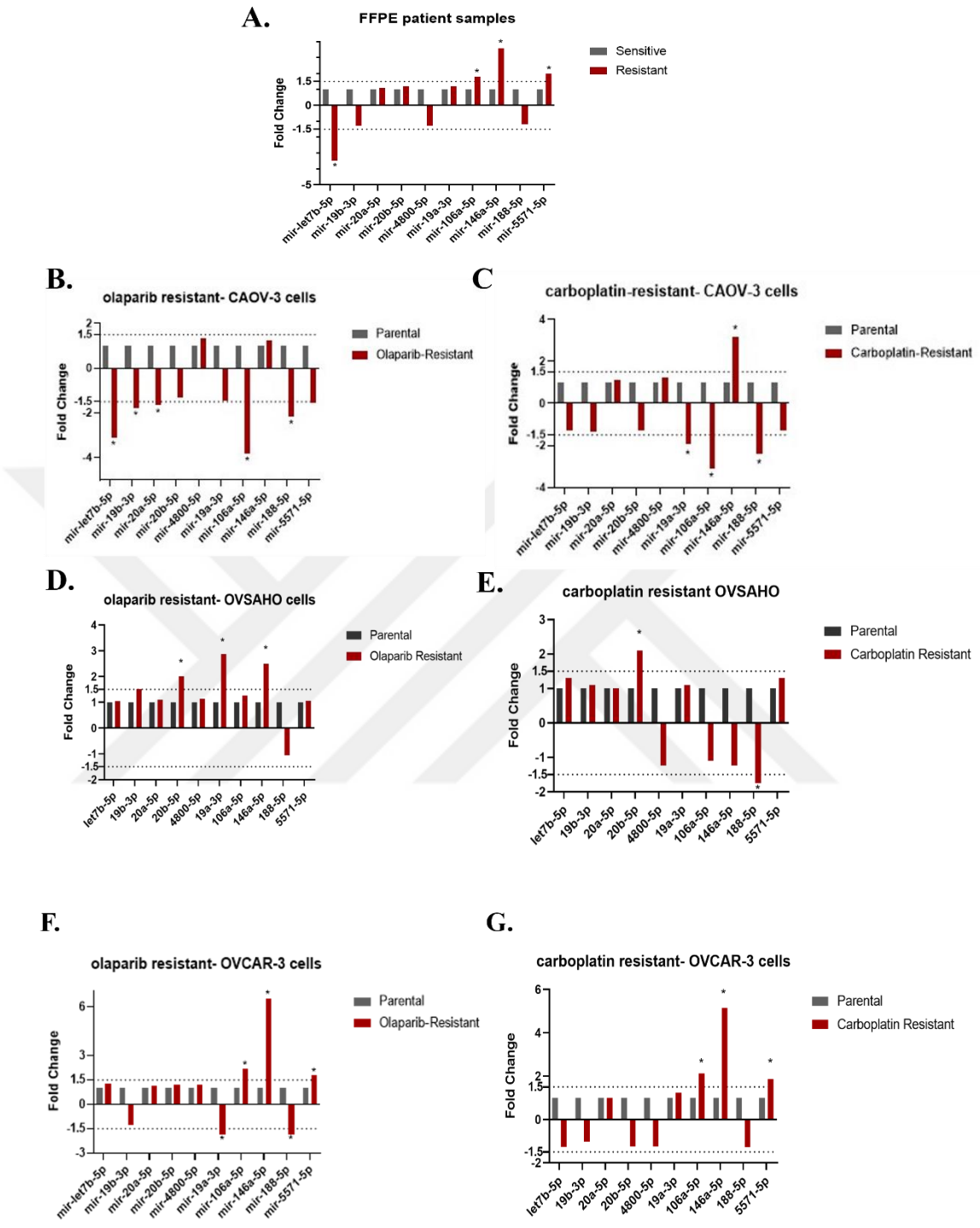


Figure 3.6: Differential expression analysis was performed by qRT-PCR assays in FFPE tissue samples(A) and *in vitro* resistant CAOV-3 (B, C), OVSAHO (D, E) and OVCAR-3 (F, G) cell samples. Results were normalized with u6 reference gene. Fold change value>|1.5| was determined as significant.

Investigating whether miRNA expression results obtained from FFPE patient samples follow a similar pattern in *in vitro* samples is important for molecular experiments to be conducted in the project. For this reason, fold change results were compared with FFPE and *in vitro* samples in **Table 3.2** Most comprehensive and significantly regulated 2 candidate miRNAs were selected for further downstream experiments included in this thesis project as **miR-*let7b-5p*** and **miR-*188-5p***.

Tablo 3.2: Differential miRNA gene expression levels were determined by microarray and qRT-PCR assays: During qPCR studies, *in vitro* and FFPE samples were evaluated within themselves. Sensitive FFPE samples for patient derived studies and parental cells for *in vitro* studies were used as control group. q-PCR analysis results for 10 candidate miRNAs selected from a total of 40 FFPE tissue samples and 3 *in vitro* resistant cell lines. The results were normalized to the U6 reference control gene. Fold change value >|1.5| were determined as significant.

Candidate miRNAs	Resistant in vitro samples Resistant/Sensitive Fold Change	qRT-PCR FFPE samples Resistant/Sensitive Fold Change	Microarray Resistant/Sensitive Fold Change
hsa-let-7b-5p	OVCAR3-olaparib: - 1,23 OVCAR3-carboplatin: - 1,26 OVSAHO -olaparib: 1,05 OVSAHO- carboplatin: 1,3 CAOV3- olaparib: 1,16 CAOV3- carboplatin: -1,44	-3,5	-1,3
hsa-miR-188-5p	OVCAR3-olaparib: -1,85 OVCAR3-carboplatin: -1,29 OVSAHO -olaparib: -1,06 OVSAHO-carboplatin: - 1,75 CAOV3-olaparib: -2,5 CAOV3-carboplatin: - 2,4	-1,2	-2,6
hsa-miR-19a-3p	OVCAR3-olaparib: -1,85 OVCAR3-carboplatin: 1,23 OVSAHO -olaparib: 2,87 OVSAHO-carboplatin: 1,1 CAOV3-olaparib: -1,44 CAOV3-carboplatin: -1,92	-1,1	1,0
hsa-miR-19b-3p	OVCAR3-olaparib: -1,26 OVCAR3-carboplatin: -1,02 OVSAHO -olaparib: 1,51 OVSAHO-carboplatin: 1,1 CAOV3-olaparib: -1,81 CAOV3-carboplatin: -1,36	-2,4	2,2
hsa-miR-20a-5p	OVCAR3-olaparib: 1,14 OVCAR3-carboplatin: 1 OVSAHO -olaparib: 1,10 OVSAHO-carboplatin: 1 CAOV3-olaparib: -1,66 CAOV3-carboplatin: 1,13	-1,8	2,1
hsa-miR-106a-5p	OVCAR3-olaparib: 2,22 OVCAR3-carboplatin: 2,12 OVSAHO -olaparib: 1,26 OVSAHO-carboplatin: -1,1 CAOV3-olaparib: -3,84 CAOV3-carboplatin: -3,13	1,4	2,1
hsa-miR-146a-5p	OVCAR3-olaparib: 6,52 OVCAR3-carboplatin: 5,15 OVSAHO -olaparib: 2,5 OVSAHO-carboplatin:- 1.4 CAOV3-olaparib: 1,26 CAOV3-carboplatin: 3,17	3,7	3,8
hsa-miR-20b-5p	OVCAR3-olaparib: 1,2 OVCAR3-carboplatin: -1,25 OVSAHO -olaparib: 2,01 OVSAHO-carboplatin: 2,1 CAOV3-olaparib: -1,31 OVCAR3-olaparib: 1,2	-2,2	2,3
hsa-miR-4800-5p	OVCAR3-olaparib: 1,22 OVCAR3-carboplatin: -1,25 OVSAHO -olaparib: 1,13 OVSAHO-carboplatin: -1,24 CAOV3-olaparib: 1,33 CAOV3-carboplatin: 1,26	-4,4	1,2
hsa-miR-5571-5p	OVCAR3-olaparib: 1,77 OVCAR3-carboplatin: 1,87 OVSAHO -olaparib: 1,06 OVSAHO-carboplatin: 1,3 CAOV3-olaparib: -1,56 CAOV3-carboplatin: -1,29	1,3	1,1

3.3 Determination of the Synergistic Effects of miRNAs on Cell Viability of Resistance Cells

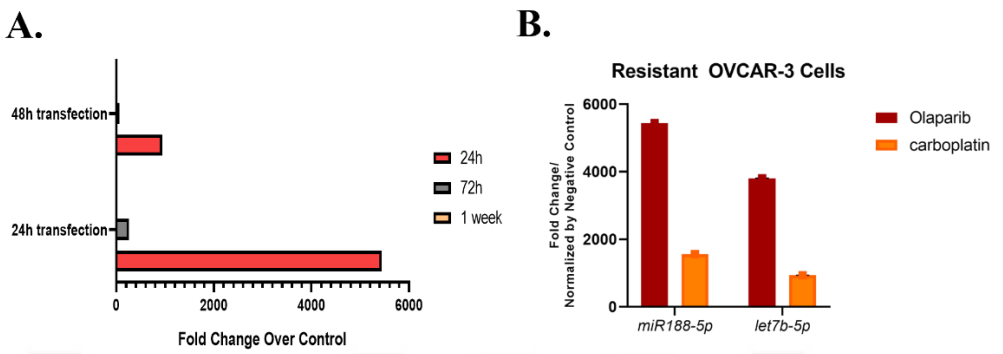


Figure 3.7: Transfection optimization assays for miRNA mimics. Representative images of expression levels of *miRlet7b* in let7b mimic transfected cells by using Lipofectamine 3000 reagent for 24h and 48h. Expression levels were determined at 24h, 72h and 1 week after 24h and 48h transfection separately **(A)**. To determine the most efficient incubation period for drug treatment, Olaparib were treated in resistant samples for 72h and 1 week after 24h let7b-mmc transfection **(B)**.

Before investigating synergistic effects of miRNAs and drugs on resistant cells, optimizing experiments of mimic transfection were performed to determine most efficient transfection and drug treatment time for resistant cells. For this purpose, olaparib and carboplatin resistant HGSOC cells were transfected with mimic of selected miRNAs by using lipofectamine 3000 reagent for 24 and 48 hours **(Figure 3.7A).** Also, to determine the most effective incubation time of drug treatment for synergistic experiments, Olaparib were treated in resistant samples for 72h and 1 week after 24h let7b-mmc transfection **(Figure 3.7B).** Depend on optimization experiments, 24h transfection with miRNA-mimics and more than 3 days of drug treatment were selected for further synergistic experiments.

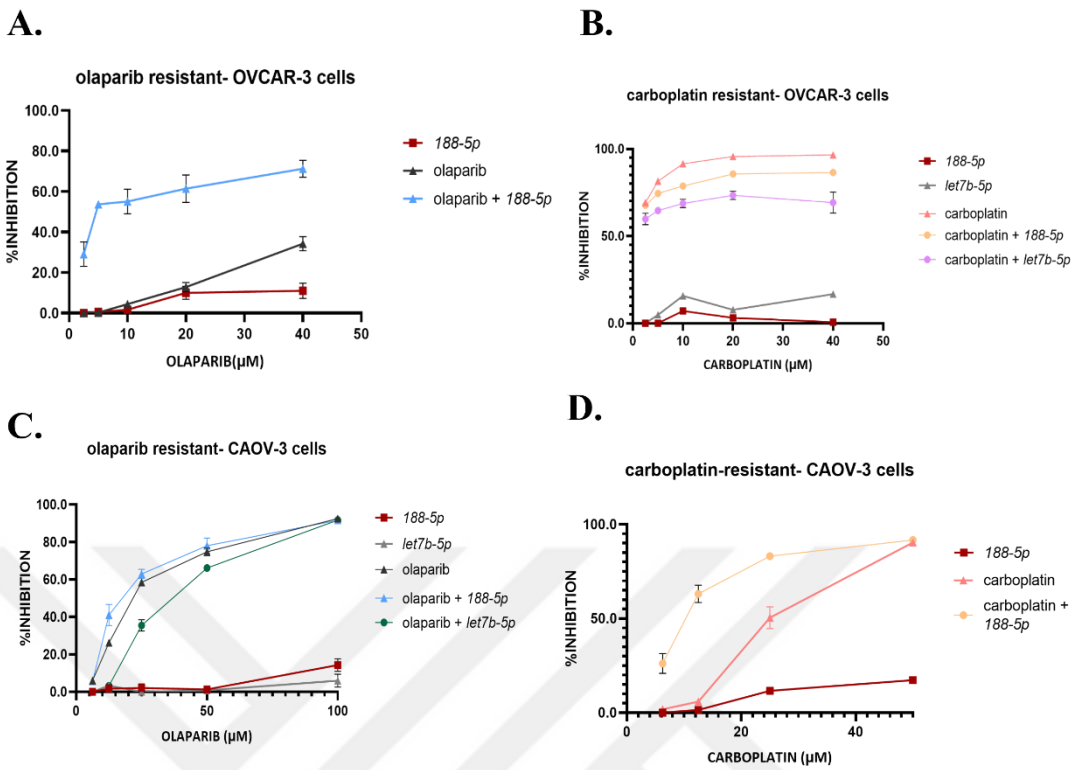


Figure 3.8: Inhibition curves of miRNA-mimic and conventional drugs analysis were examined by SRB cell viability assay in mimic transfected olaparib resistant OVCAR-3 (A), carboplatin resistant OVCAR-3 (B), olaparib resistant CAOV-3 (C), carboplatin resistant CAOV-3 (D) cell samples. Results were compared with NC transfected resistant cells.

After transfection, transfected cells with miRNA mimic were treated with chemotherapeutic agents currently used clinically for ovarian cancer treatment (Olaparib and carboplatin) to perform a synergy analysis by SRB cell viability assay. Control samples were used as negative control in these experiments. The following treatments were applied to each of the resistant cell groups; Olaparib, DMSO negative control (Since Olaparib are dissolved in DMSO), miRNA mimic +DMSO, miRNA mimic +Olaparib. The inhibition curves and bar graphs of each treatment were showed **(Figure 3.8 and 3.9)** and the synergistic effects of the selected miRNA with the drugs were determined (**Figure 3.9**).

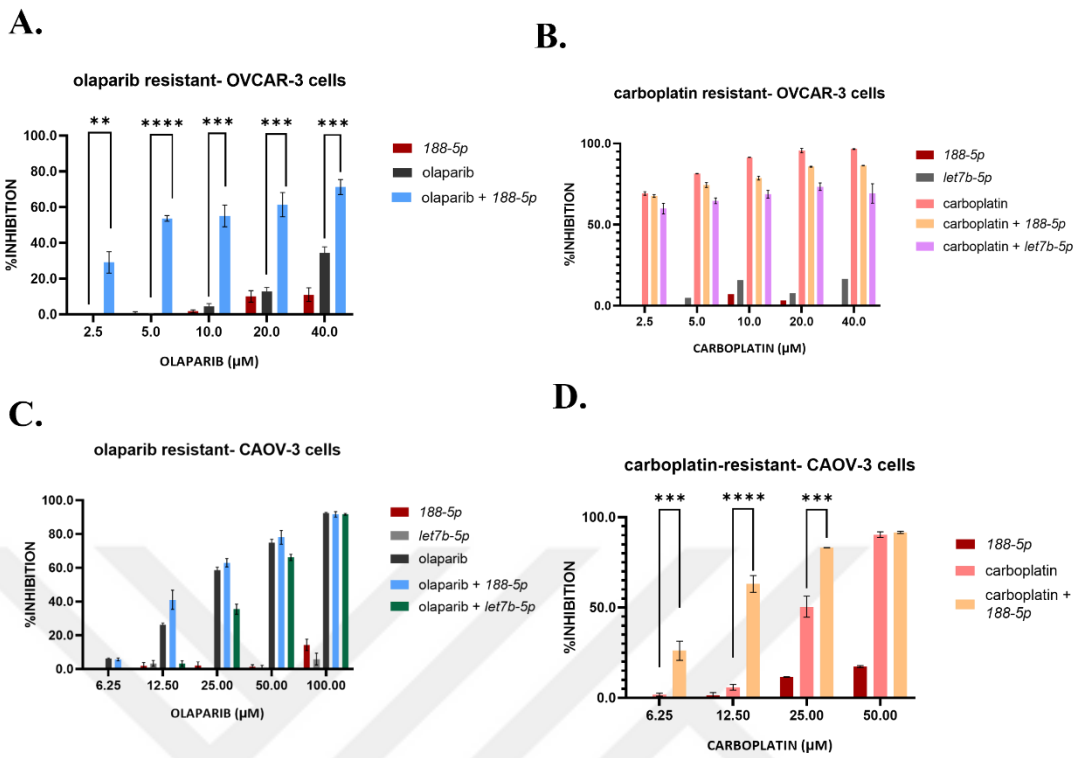


Figure 3.9: Inhibition bar graphs of miRNA-mimic and conventional drugs analysis were examined by SRB cell viability assay in mimic transfected olaparib resistant OVCAR-3 (A), carboplatin resistant OVCAR-3 (B), olaparib resistant CAOV-3 (C), carboplatin resistant CAOV-3 (D) cell samples. Results were compared with NC transfected resistant cells. Significancy were determined using non-parametric multiple t-test.

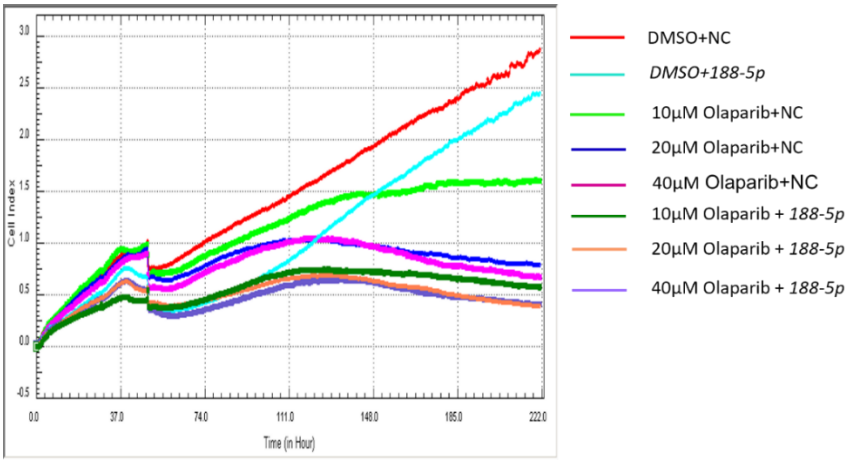


Figure 3.10: Representative xCELLigence-real time Cell Index results for the negative control and *188-5p* transfected OVCAR-3 Olaparib resistant cells under treatment with 10 μM, 20 μM, 40 μM and DMSO control for 1 week. Cells were transfected with NC, and miRNA for 24 hours after seeding, then treated with determined concentrations of Olaparib.

The results were compared with monotherapy and combination therapy with 2 selected miRNAs, and the most effective treatment strategy was determined as miR-*188-5p* on Olaparib resistant OVCAR-3 cells for further experiments. To determine the most effective Olaparib dose xCelligence cell viability assay was performed for miR*188-5p* transfected- Olaparib resistant OVCAR-3 cells. xCelligence real time cell viability assay results were showed in **Figure 3.10**. In addition, Combinational Index values were calculated for negative control and *188-5p* transfected OVCAR-3 Olaparib resistant cells under treatment with 5 µM, 10 µM, 20 µM and control for 5 days **(Tablo 3.3).** Depend on these results, we selected 10 µM of Olaparib for 5 days incubation as most synergistic dose for further downstream investigations.

Tablo 3.3: Combinational Index analysis of negative control and *188-5p* transfected OVCAR-3 Olaparib resistant cells under treatment with 5 µM, 10 µM, 20 µM and control for 5 days. Values <1 were determined as synergistic with Olaparib.

Olaparib resistant - OVCAR-3 cells		*188-5p*	*188-5p*	*188-5p*	Negative Control
OLAPARIB	40 µM	0.35			1.00
	20 µM		0.07		1.00
	10 µM			**0.02**	1.00
	0	1.00	1.00	1.00	

3.4 Determination of the Synergistic Effects of miRNAs on Cell Viability of ex-vivo Cell lines

In this study, the effect of the combination therapy of miR*188-5p* mimic product and Olaparib on sensitive and resistant *ex vivo* patient samples was investigated. For this purpose, *ex vivo* patient derived cell lines were generated and treatment response of *ex vivo* samples against Olaparib treatment were determined. Next, patients divided into two subgroups depend on their IC_{50} values and showed in **Table 3.4**.

Table 3.4: IC_{50} values of *ex vivo* patients.

PATIENT NO	TREATMENT	IC_{50}
#1	olaparib	174.7 µM± 30.8
#2	olaparib	96.5 µM± 7.3
#3	olaparib	43.4 µM± 5.1
#4	olaparib	35.3 µM± 1.4

MiR-*188-5p* expression levels of sensitive and resistant patients were identified by qPCR experiment. In parallel with *in vitro* results, resistant *ex vivo* patient sample's expression levels were found significantly downregulated (**Figure 3.11**).

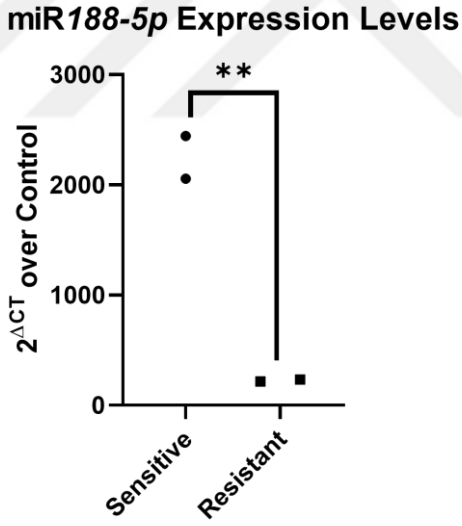


Figure 3.11: Fold change graph of mimic expression of *ex vivo* cell samples transfected with negative control and *miR188-5p* mimic products. Statistical analysis was made with student t test, ** p=0.0091.

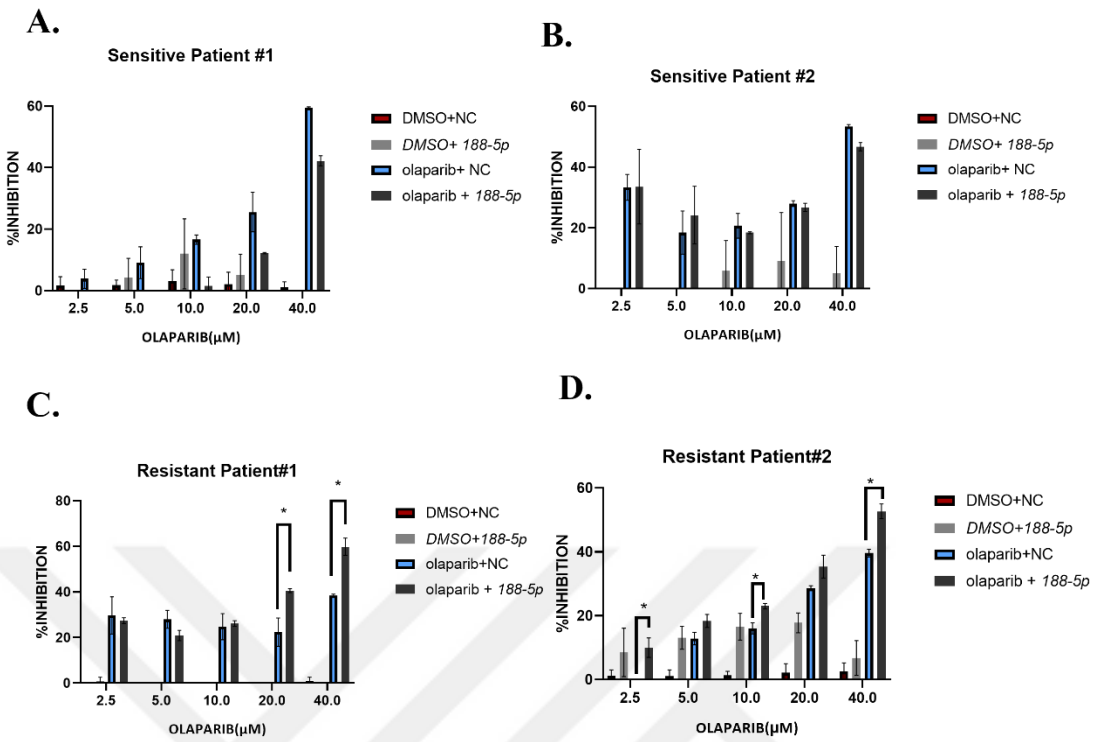


Figure 3.12: Inhibition bar graphs of *ex vivo* cells transfected with miR-*188-5p* mimic and negative control have been showed. Significancy were determined using non-parametric multiple t-test.

As shown in the inhibition curve graphs **(Figure 3.12)**, the viability of resistant *ex vivo* cell samples transfected with the miR*188-5p* mimic product decreased, indicating that resistant cells became sensitive to the effects of miRNA mimics. However, in sensitive patients, the level of cell viability remained unchanged. Additionally, the IC_{50} concentrations of Olaparib for *ex vivo* patients transfected with mimic product and negative control were calculated. The comparative IC_{50} tables **(Table 3.5)** demonstrate that the IC_{50} values of *ex vivo* cells significantly decreased because of the combination therapy of mimic and Olaparib.

Table 3.5: IC_{50} values for *ex vivo* resistant patients with monotherapy and combination therapy

RESISTANT	TREATMENT	IC_{50}
#1	NC+ olaparib	174.7 µM± 30.8
	1885p+olaparib	25.2 µM± 0.4
#2	NC+ olaparib	96.5 µM± 7.3
	1885p+ olaparib	44.6 µM± 0.7

Additionally, a combination index calculation was performed on the results, and the outcomes are presented in **Table 3.6** and **3.7.** The combination index values indicate the presence of synergistic interactions in resistant patients. The viability of *ex vivo* resistant patients' cells decreased with combination therapy compared to monotherapy, and their IC50 values fell within the same range as the sensitive patient group. Thus, the role of miR1885p in overcoming resistance has been demonstrated.

Table 3.6: **The synergy index calculation table for the combination therapy of miR*188-5p* in resistant *ex vivo* cells (patient no. 1) is shown.** The results were normalized with DMSO. Results identified as <1 are considered to exhibit a synergistic effect.

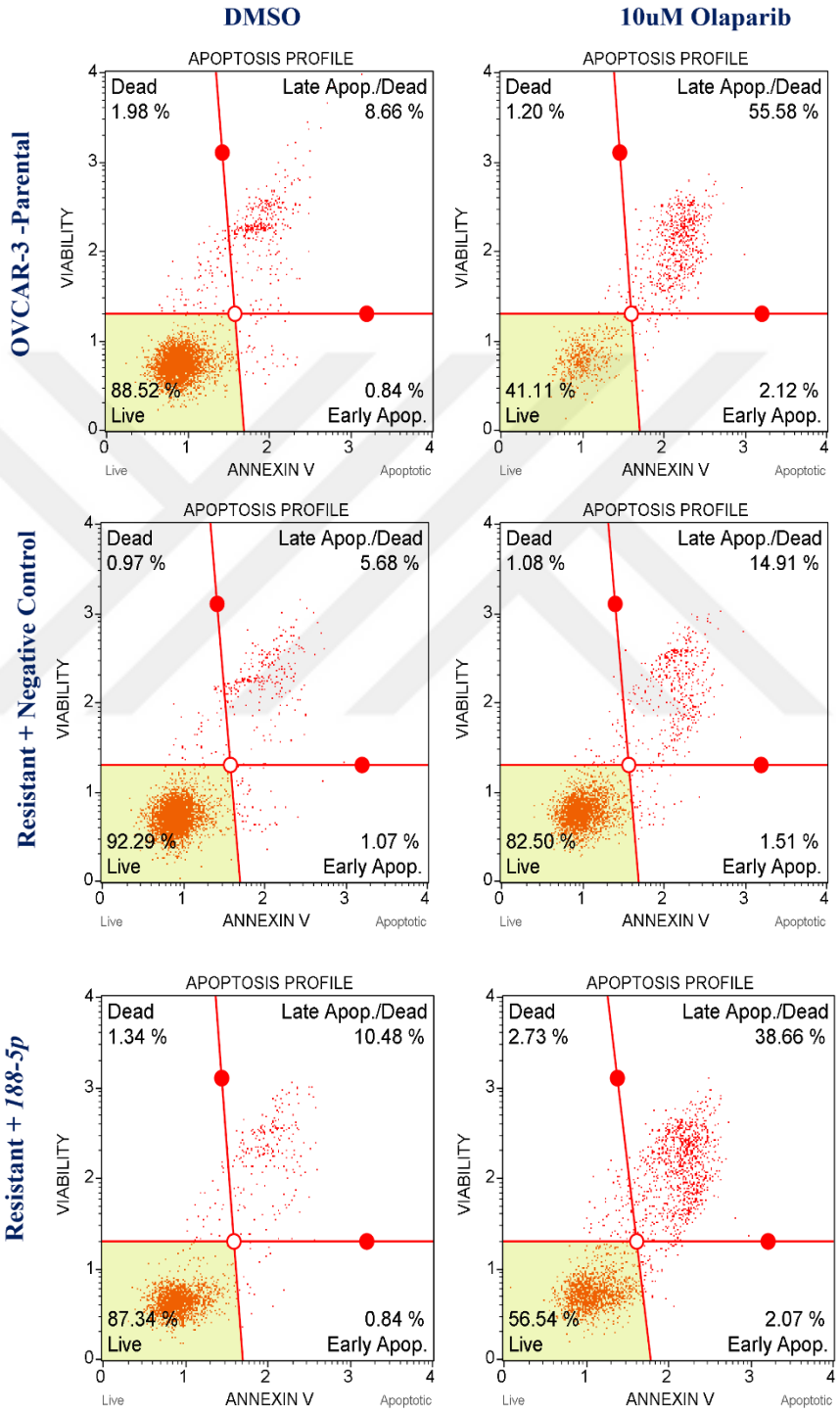
Resistant patient#1		*188-5p*	*188-5p*	*188-5p*	Negative Control
OLAPARIB	40	0.26			1.00
	20		0.14		1.00
	10			0.69	1.00
	0	1.00	1.00	1.00	0

Table 3.7: The synergy index calculation table for the combination therapy of miR*188-5p* in resistant *ex vivo* cells (patient no: 2) is provided. The results were normalized with DMSO. Results identified as <1 are considered to exhibit a synergistic effect.

Resistant patient#2		*188-5p*	*188-5p*	*188-5p*	Negative Control
OLAPARIB	40	0.48			1.00
	20		0.96		1.00
	10			1.19	1.00
	0	1.08	0.86	0.87	0

3.5 Investigation of the Synergistic Effects of miR188-5p on Cell Cycle and Apoptosis and EMT Mechanisms of Resistance Cells

A.



B.

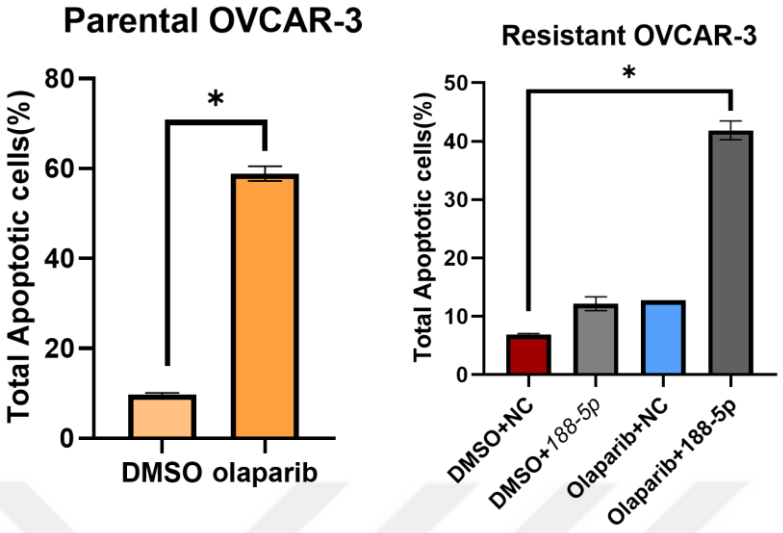
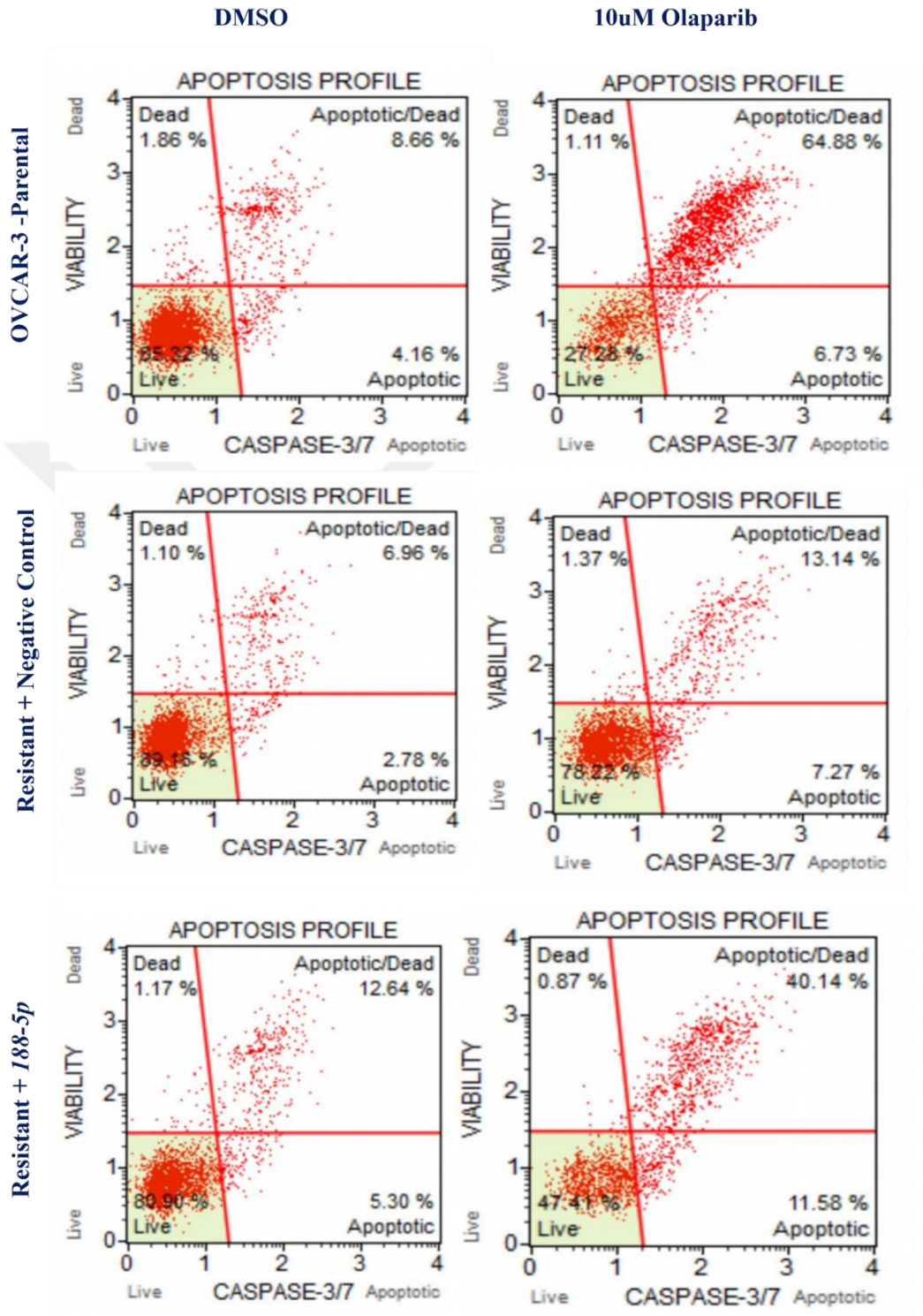


Figure 3.13: Representative results of MUSE Annexin-V (A) in parental OVCAR-3, NC transfected and miR*188-5p* transfected olaparib resistant OVCAR-3 cells with 10µM Olaparib treatment at 5 days. DMSO was used as a negative control. The results demonstrate an increase in total apoptotic cells for miRNA transfected cells. **(B)** Representative results for the quantitative measurement of Annexin-V staining by flow cytometry readings for same experiment group.

To determine the changes on apoptosis profile of miRNA transfected resistant samples, mimic miR-*188-5p* transfected Olaparib resistant OVCAR-3 cells, Negative control transfected resistant OVCAR-3 cells and parental OVCAR-3 cells were treated with 10µM Olaparib treatment at 5 days. DMSO was used as a negative control. After 5 days cells were stained with apoptosis markers; Annexin V **(Figure 3.13)** and Caspase 3/7 **(Figure 3.14)** and analyzed by MUSE device.

A.


DMSO
10uM Olaparib
OVCAR-3 -Parental
Resistant + Negative Control
Resistant + 188-5p
APOPTOSIS PROFILE
Dead
Apoptotic/Dead
Live
Apoptotic
VIABILITY
CASPASE-3/7
1.86 %
8.66 %
85.32 %
4.16 %
1.11 %
64.88 %
27.28 %
6.73 %
1.10 %
6.96 %
89.16 %
2.78 %
1.37 %
13.14 %
78.22 %
7.27 %
1.17 %
12.64 %
80.90 %
5.30 %
0.87 %
40.14 %
47.41 %
11.58 %

B.

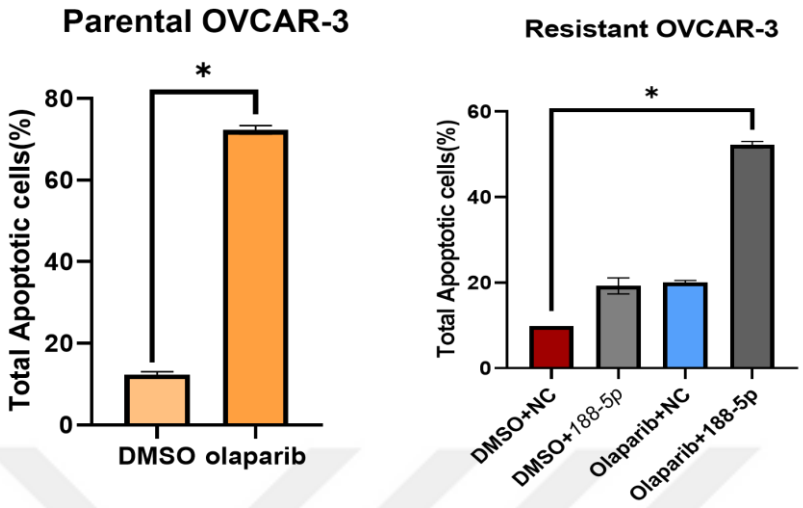


Figure 3.14: Representative results of Caspase-3/7-AAD apoptosis assay (A) in parental OVCAR-3, NC and miR*188-5p* transfected Olaparib resistant OVCAR-3 cells with 10µM Olaparib treatment at 5 days. DMSO was used as a negative control. The results demonstrate an increase in total apoptotic cells for miRNA transfected cells. **(B)** Representative results for the quantitative measurement of Caspase-3/7-AAD staining by flow cytometry readings for same experiment group.

When we compared with the NC transfected cells, we determined the increase in total apoptotic cells for miRNA transfected- resistant cells. Furthermore, we aimed to investigate cell cycle level changes of miR*188-5p* transfected-Olaparib resistant OVCAR-3 cells in compared with the control group. For this purpose, cells were treated with 10µM Olaparib for 5 days cells and fixed. Fixed cells were stained with Propidium-Iodide (PI) solution and analyzed by flow cytometry. The results demonstrate increase in subG1 phase in miRNA mimic transfected sample in compared with negative control group **(Figure 3.15).**

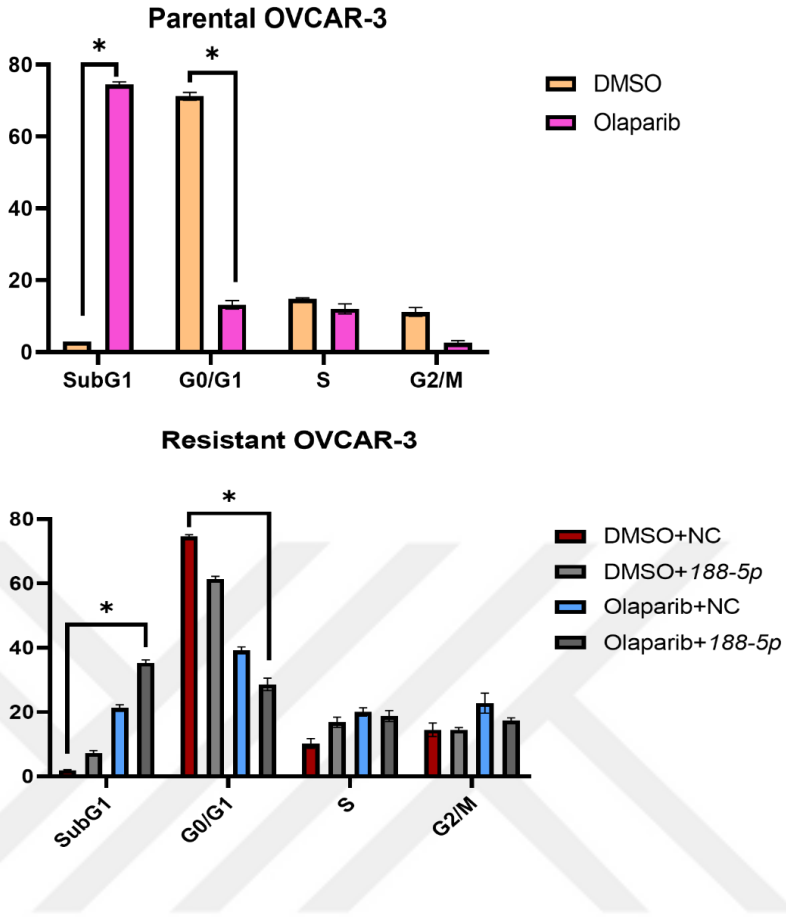
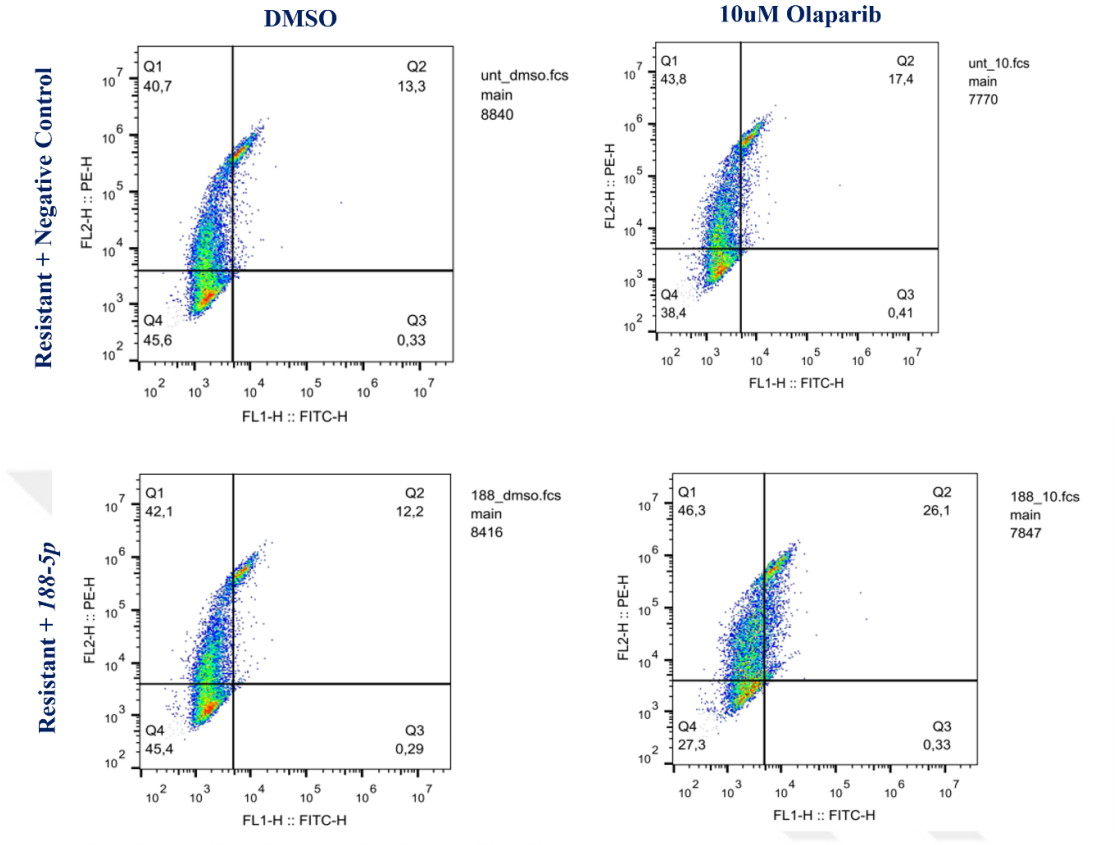


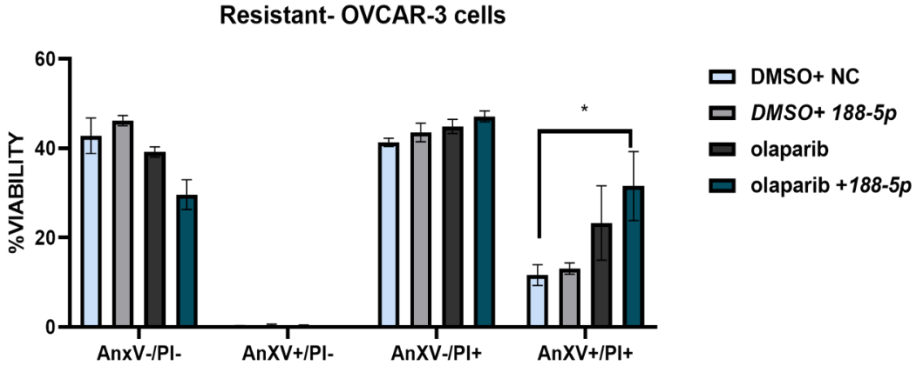
Figure 3.15: Representative results for the quantitative measurement of cell cycle phases using PI staining and flow cytometry readings in parental OVCAR-3, NC-transfected and miR*188-5p* transfected Olaparib resistant OVCAR-3 cells with 10µM Olaparib treatment at 5 days. DMSO was used as a negative control. The results demonstrate an increase in subG1 phase in miRNA mimic transfected sample in compared with control group.

We confirmed the apoptosis profile of the Olaparib resistant OVCAR-3 cells under treatment with monotherapy and combinational therapy with *188-5p* and 10µM Olaparib for 5 days using Annexin V and PI staining and flow cytometry readings and Immunofluorescence experiment.

A.


DMSO
10uM Olaparib
Resistant + Negative Control
Resistant + 188-5p
Q1 40,7
Q2 13,3
Q4 45,6
Q3 0,33
unt_dmso.fcs
main
8840
Q1 43,8
Q2 17,4
Q4 38,4
Q3 0,41
unt_10.fcs
main
7770
Q1 42,1
Q2 12,2
Q4 45,4
Q3 0,29
188_dmso.fcs
main
8416
Q1 46,3
Q2 26,1
Q4 27,3
Q3 0,33
188_10.fcs
main
7847
FL1-H :: FITC-H
FL2-H :: PE-H

B.


Resistant- OVCAR-3 cells
%VIABILITY
60
40
20
0
AnxV-/PI-
AnXV+/PI-
AnXV-/PI+
AnXV+/PI+
*
DMSO+ NC
DMSO+ 188-5p
olaparib
olaparib +188-5p

C.

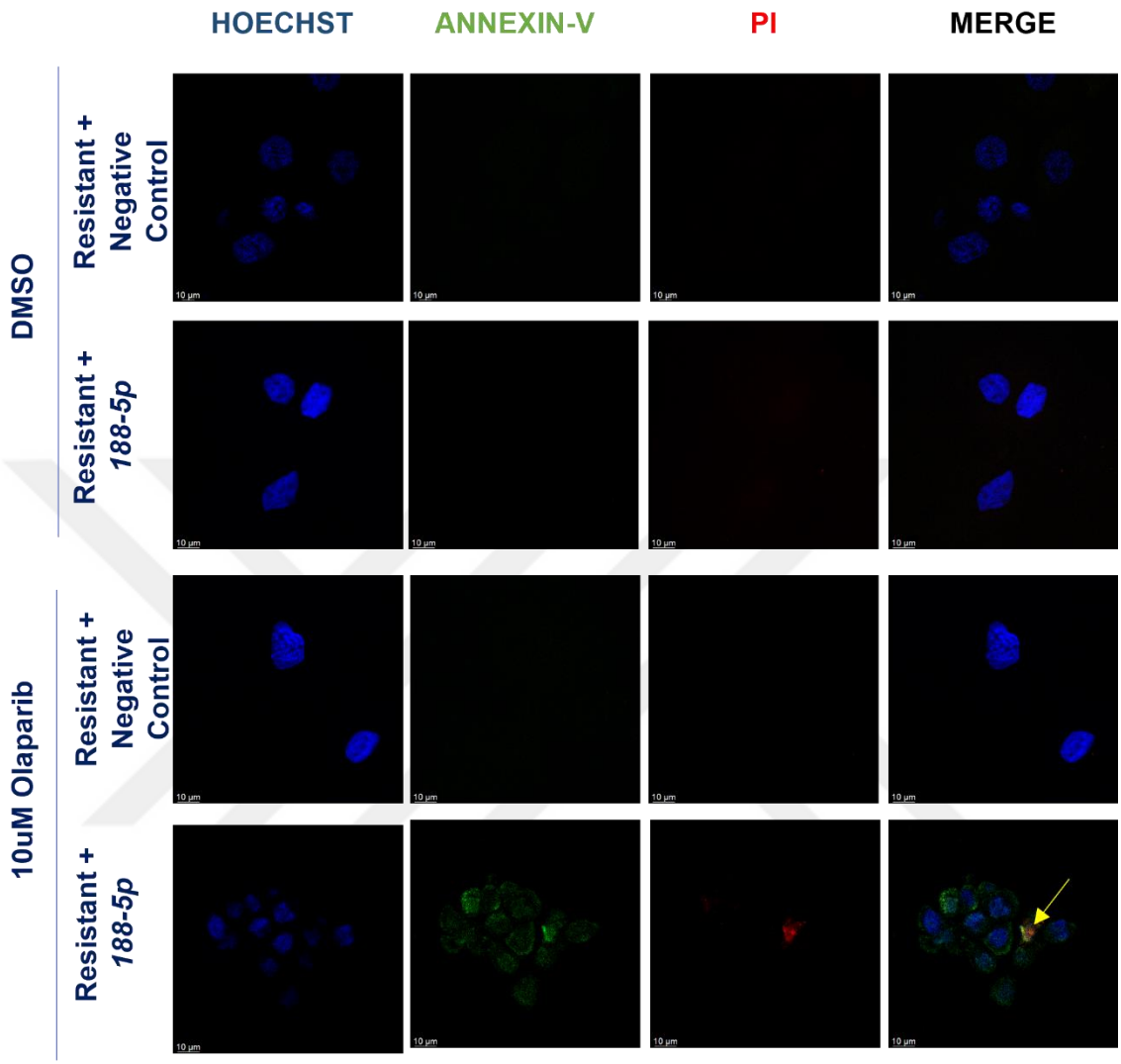


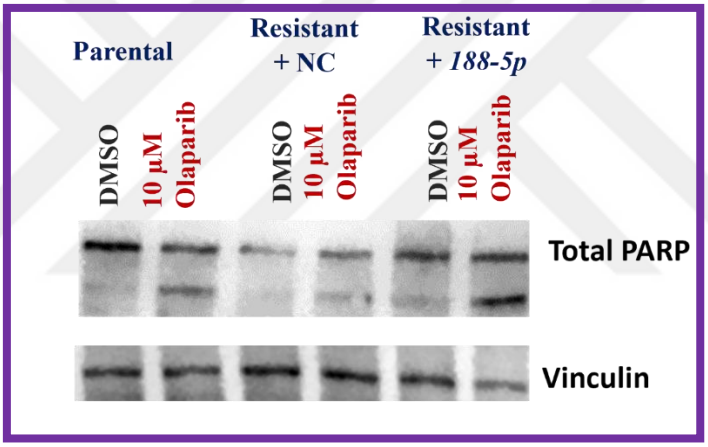
Figure 3.16: Representative results of the apoptosis profile of the Olaparib resistant OVCAR-3 cells under treatment with monotherapy and combinational therapy with *188-5p* and 10µM Olaparib for 5 days using Annexin V and PI staining and flow cytometry readings (A). Representative results of **the** quantitative measurement of staining **Annexin V and PI** by flow cytometry readings for same experiment group (* P=0.004) (B). Microscopic images of the Annexin V and PI staining via Immunofluorescence staining **(C)**. Images were taken by confocal microscopy, original magnification 40X. Scale bar = 10 µM. **The results demonstrate an increase in apoptosis level in *188-5p* transfected resistant in compared with control group after Olaparib treatment.**

Representative results of the quantitative measurement of staining Annexin V and PI by flow cytometry readings and microscopic images were shown in **Figure 3.16**. The results

demonstrate an increase in apoptosis level in *188-5p* transfected resistant in compared with control group after Olaparib treatment.

Various proteins responsible for cell death and the epithelial-mesenchymal transition (EMT) mechanism (total PARP and cleaved PARP, cleaved and total caspase 8, phosphorylated and total ATR, Akt, p-Akt; N-cadherin, E-cadherin, SNAIL, SLUG) were compared in parental and resistant OVCAR-3 cells treated with 10 µM Olaparib and DMSO as monotherapy, and resistant OVCAR-3 cells treated with Olaparib, DMSO, and olaparib+miRNA *188-5p* mimic in combination. The method specified in the project was followed for Western blot experiments. Calnexin, vinculin, beta-actin, and alpha-tubulin were used as housekeeping proteins.

A.



B.

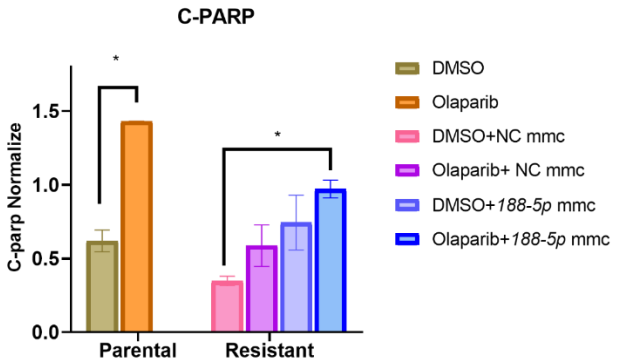
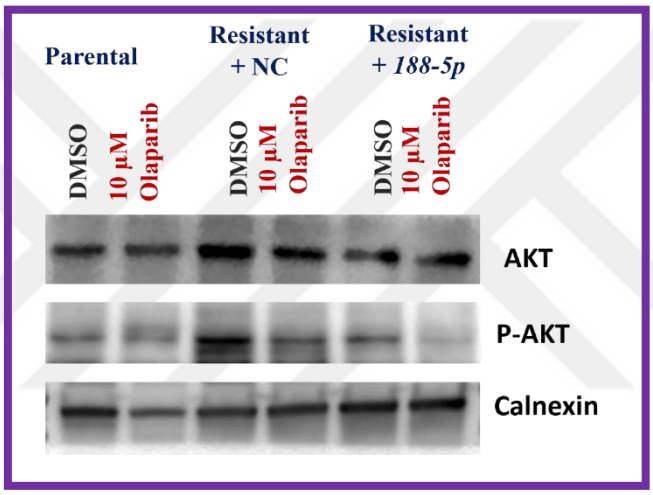


Figure 3.17: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using Total and cleaved PARP antibodies are shown by Western blot experiments. Vinculin was used as the housekeeping protein. **B)** The results were quantitatively analyzed using the Image J program. Statistical significance

was determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis.

When evaluating the effect of miRNA *188-5p* and Olaparib combination therapy on cell death, resistant sample group treated with combination therapy showed significantly higher levels of cleaved PARP protein compared to the monotherapy **(Figure 3.17).** These assessments suggest that miRNA-drug combination therapy enhances cell death, thereby reducing resistance.

A.



B.

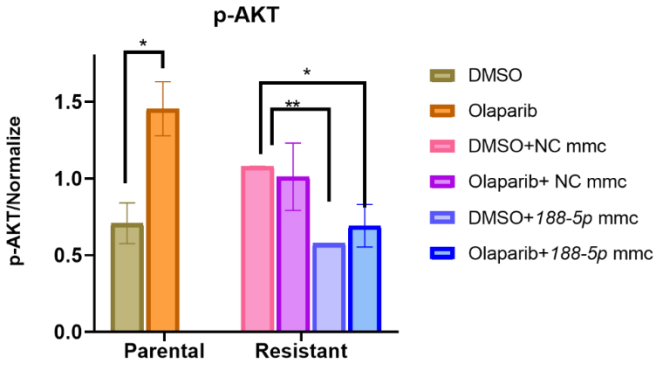
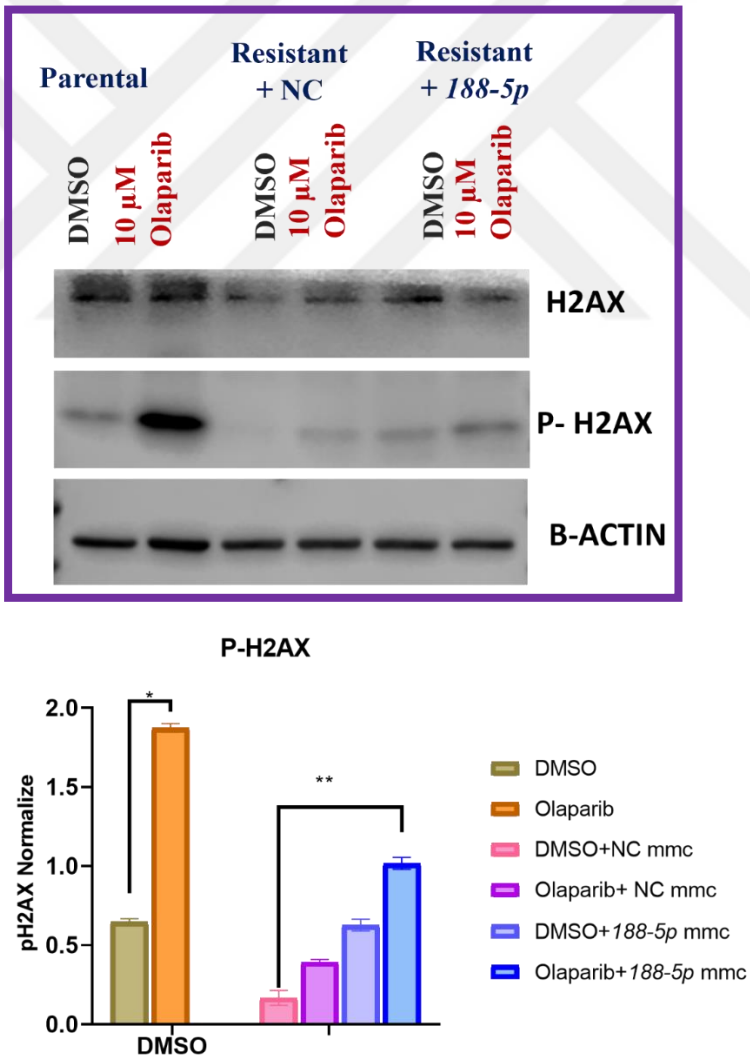


Figure 3.18: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using Akt and p-Akt antibodies are shown by Western blot experiments. Calnexin was used as the housekeeping protein**. B)** The results were quantitatively analyzed using the Image J program. Statistical significance was

determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis.

The level of phosphorylated AKT protein is significantly decreased in the resistant sample groups that received combination therapy compared to the control. Phosphorylated AKT protein, the active form of AKT, is associated with cell survival mechanisms, and a decrease in its sensitive expression is expected. Therefore, these results suggest that the target miRNA (*188-5p*) may exert its effect on re-sensitization mechanism through the AKT pathway. This finding is presented as an important discovery supporting the objectives of this project **(Figure 3.18).**

A.



C.

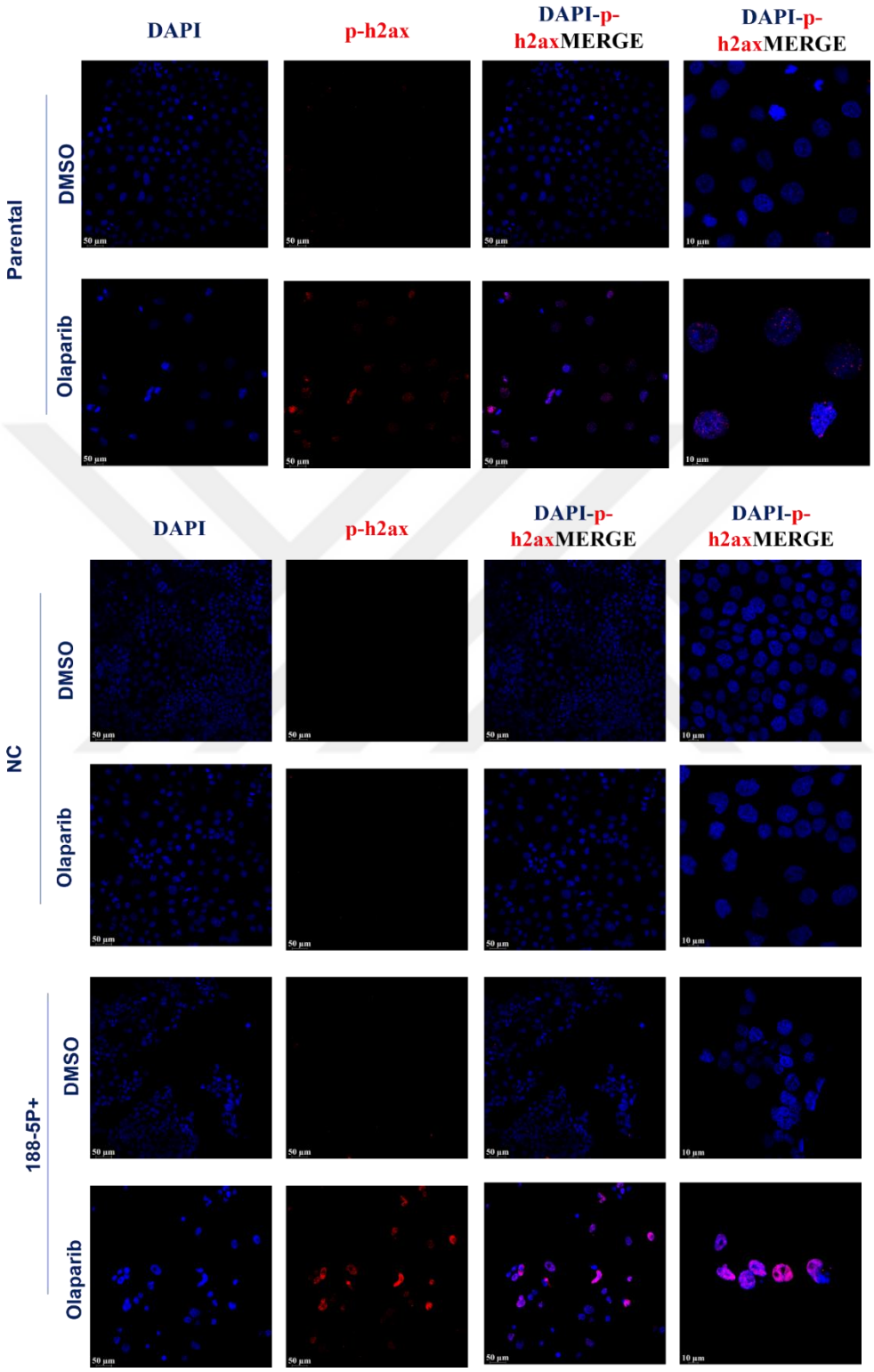
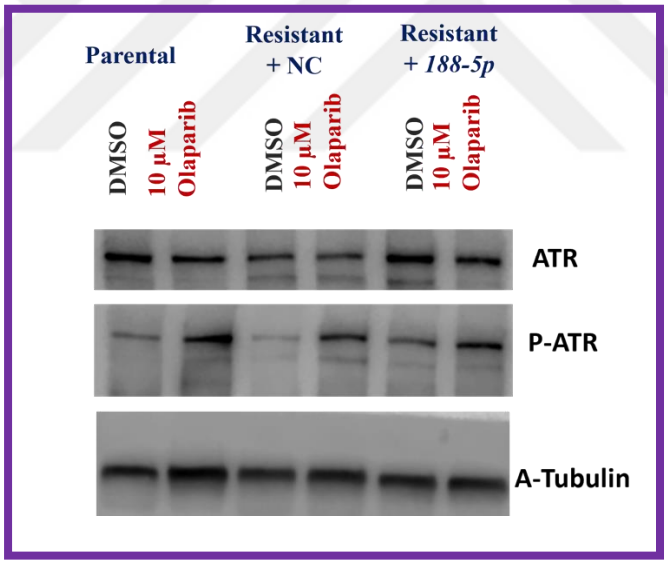


Figure 3.19: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using H2AX and p- H2AX antibodies are shown by Western blot experiments. Calnexin was used as the housekeeping protein. **B)** The results were quantitatively analyzed using the Image J program. Statistical

significance was determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis. **C)** Images were taken by confocal microscopy, original magnification 40X. Scale bar = 10 µM. **The results demonstrate an increase in p-H2AX level in *188-5p* transfected resistant in compared with control group after Olaparib treatment.**

In ovarian cancer, the importance of DNA damage repair mechanisms and the proteins involved in these processes are significant. H2AX and ATR play critical roles in detecting and repairing DNA damage. H2AX is a phosphorylated form of H2A, a histone protein that detects damage in the double-stranded DNA and signals the cell for repair. Upon detection of DNA damage, H2AX is rapidly phosphorylated and localized to the site of damage, facilitating the activation of DNA repair mechanisms and the resolution of damage.

A.



B.

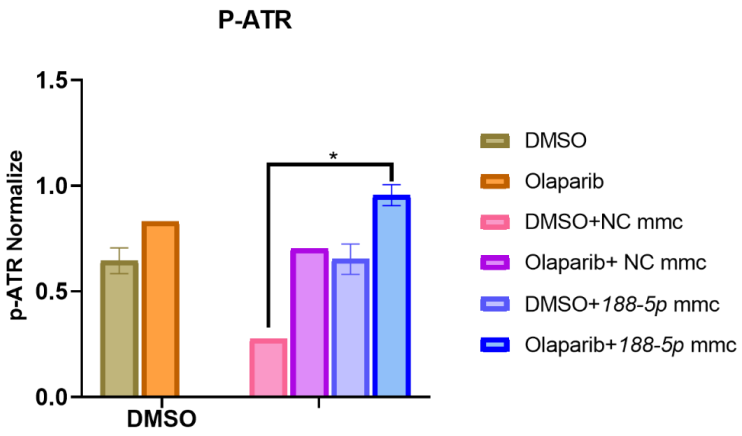
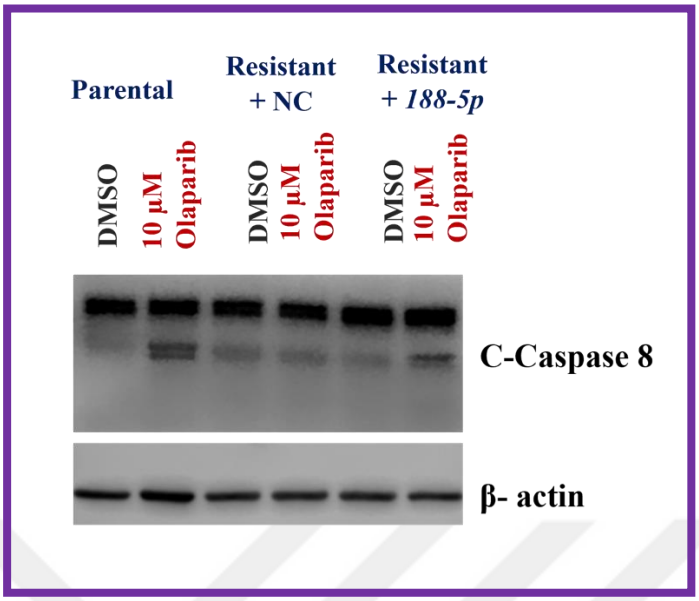


Figure 3.20: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using ATR and p- ATR antibodies are shown by Western blot experiments. Calnexin was used as the housekeeping protein**. B)** The results were quantitatively analyzed using the Image J program. Statistical significance was determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis.

ATR (Ataxia Telangiectasia and Rad3-related) protein kinase plays a crucial role in DNA damage sensing and signal transduction. Activated ATR halts the cell cycle, allowing for the repair of damaged DNA, or induces cell apoptosis in cases where the damage is irreparable. In resistant cells, DNA damage detection and repair mechanisms often show excessive activation or increased expression levels of critical proteins in these mechanisms. In our results, within synergistic combination with mimic and *188-5p*, significantly increased level of p-ATR and p-h2AX proteins were showed. We conclude these results as; synergy can enhance the ability of cancer cells exposed to chemotherapeutic agents to detect and repair DNA damage **(Figure 3.19 and 3.20).**

A.



B.

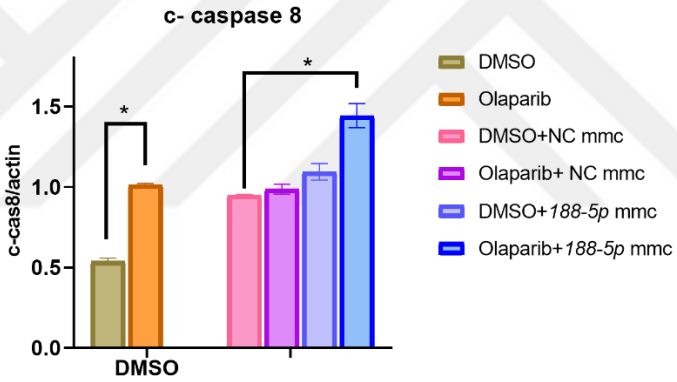


Figure 3.21: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using total and cleaved caspase-8 antibodies are shown by Western blot experiments. Calnexin was used as the housekeeping protein. **B)** The results were quantitatively analyzed using the Image J program. Statistical significance was determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis.

As shown in **Figure 3.21**, it was determined that the amount of cleaved caspase 8 significantly increased in resistant OVCAR-3 cells treated with combination therapy. Caspase 8 is a crucial initiator caspase involved in the extrinsic apoptosis mechanism induced by extracellular signals. Its cleaved form is the active form, and it was demonstrated in this study that it was activated to a higher level in the miRNA and Olaparib synergy in combination with parental cells.

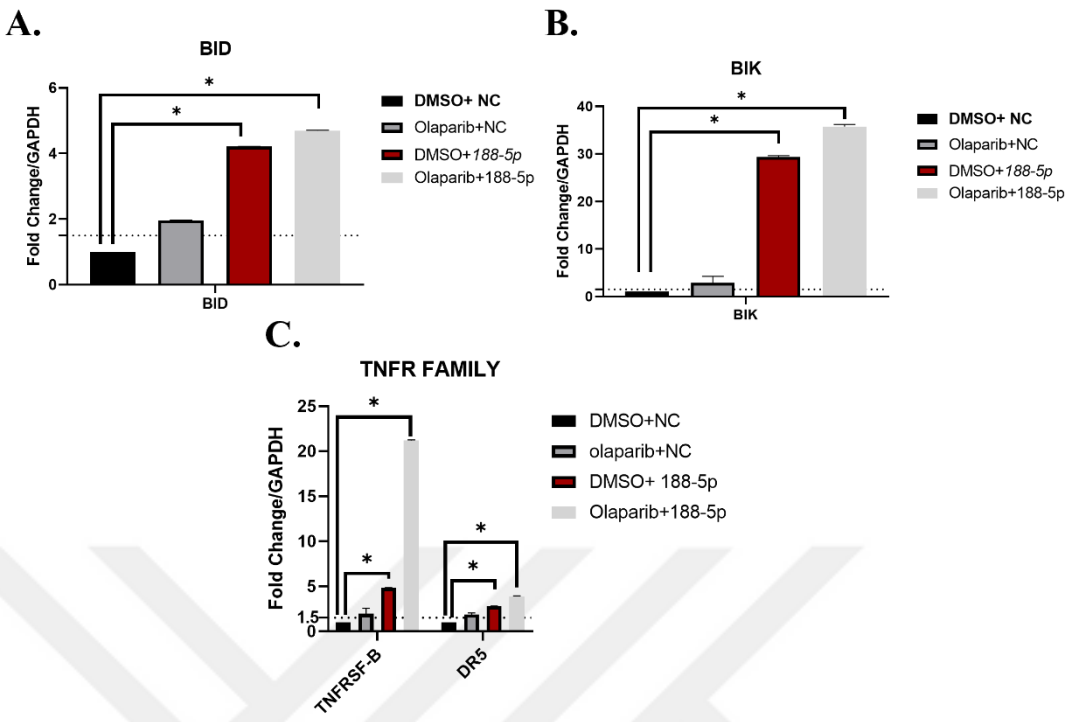
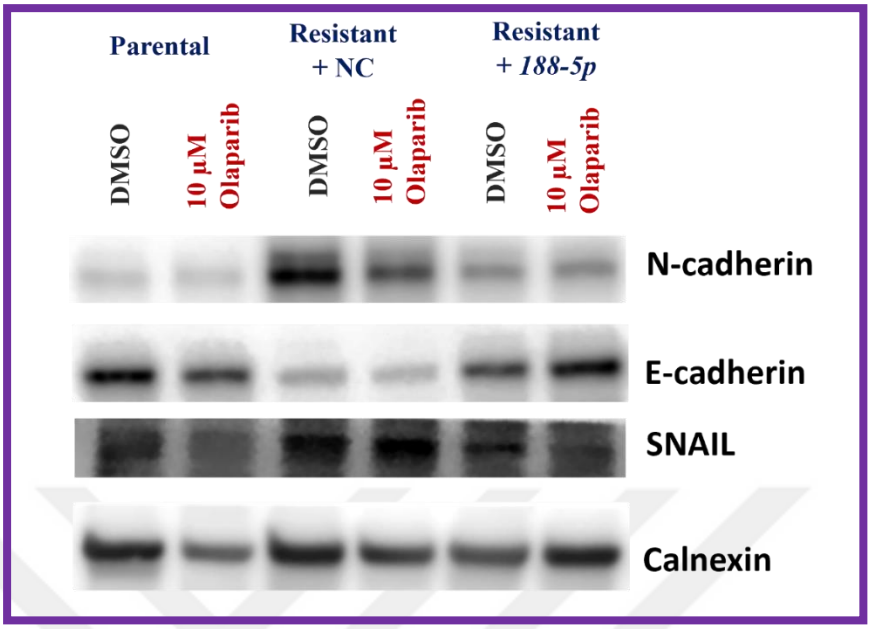


Figure 3.22: mRNA expression levels of pro-apoptotic proteins and TNF receptor family were determined in resistant cell by q-RT PCR.

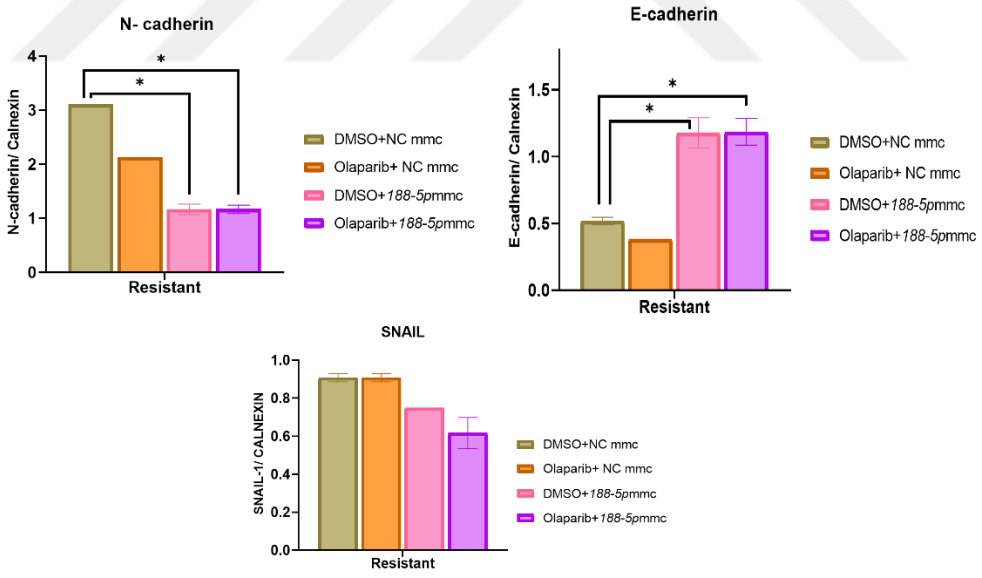
Pro-apoptotic signals, including BAX, BID, and death receptors of the TNF (tumor necrosis factor) family, play crucial roles in initiating programmed cell death, known as apoptosis. BAX and BID are members of the Bcl-2 protein family, which regulate mitochondrial membrane permeabilization, a key step in apoptosis.

Death receptors belong to the TNF receptor superfamily and are located on the cell membrane. Upon binding to their respective ligands, such as TNF-α, Fas ligand (FasL), or TRAIL (TNF-related apoptosis-inducing ligand), death receptors initiate the extrinsic apoptosis pathway. This pathway activates activator caspase 8 directly through receptor clustering and recruitment of adaptor proteins, leading to cell death. In our qPCR experiment results, BAX, BID and BIK gene levels were significantly increased (fold change value>1.8 determined as significant) in synergistically treated resistant cells. Also, TNF receptor family members; NF-KB1, DR5 and TNFR-SH gene levels were significantly upregulated upon mimic transfection and combinational therapy with Olaparib. These results were found correlated with caspase-8 protein level analysis.

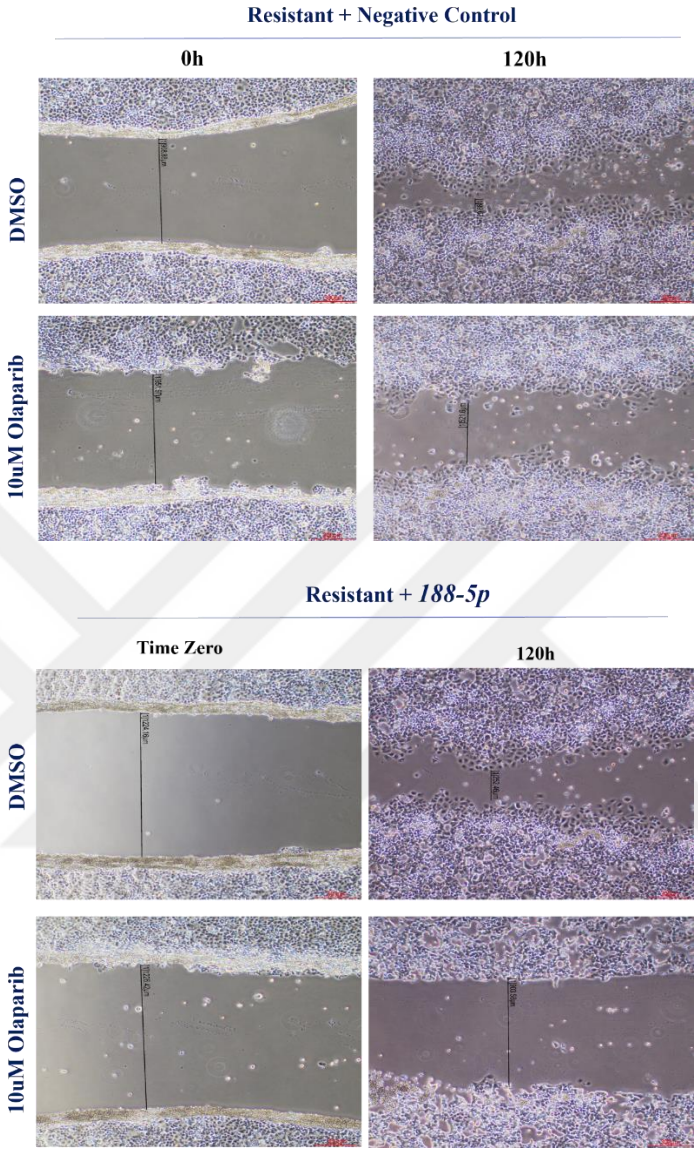
A.


Parental
Resistant + NC
Resistant + 188-5p
DMSO
10 µM Olaparib
DMSO
10 µM Olaparib
DMSO
10 µM Olaparib
N-cadherin
E-cadherin
SNAIL
Calnexin

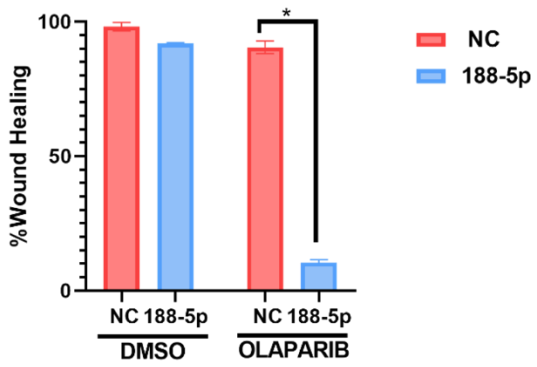
B.


N- cadherin
N-cadherin/ Calnexin
Resistant
DMSO+NC mmc
Olaparib+ NC mmc
DMSO+188-5pmmc
Olaparib+188-5pmmc
E-cadherin
E-cadherin/ Calnexin
Resistant
DMSO+NC mmc
Olaparib+ NC mmc
DMSO+188-5pmmc
Olaparib+188-5pmmc
SNAIL
SNAIL-1/ CALNEXIN
Resistant
DMSO+NC mmc
Olaparib+ NC mmc
DMSO+188-5pmmc
Olaparib+188-5pmmc

C.


Resistant + Negative Control
0h
120h
DMSO
10uM Olaparib
Resistant + 188-5p
Time Zero
120h
DMSO
10uM Olaparib

D.


100
50
0
%Wound Healing
*
NC
188-5p
NC 188-5p
NC 188-5p
DMSO
OLAPARIB

E.

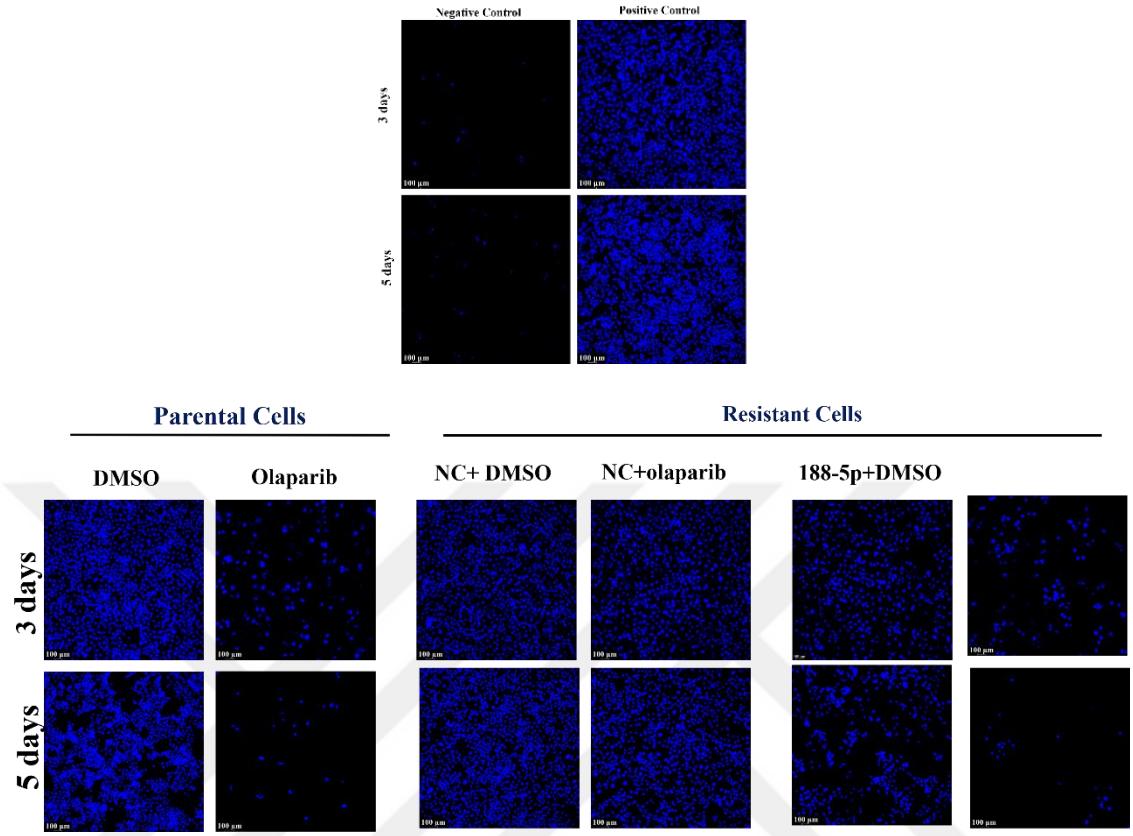


Figure 3.23: A) Protein level changes in parental and resistant OVCAR-3 cell lines treated with combination and monotherapy using N-cadherin, E-cadherin and SNAIL antibodies are shown by Western blot experiments. Calnexin was used as the housekeeping protein. **B)** The results were quantitatively analyzed using the Image J program. Statistical significance was determined using the student T test ($p<0.05$). Best representative images were selected from n=2 replicates, and all target proteins were normalized to their own housekeeping control during the quantification analysis. **C) Wound healing assay.** Representative light microscope images of WH assays for resistant OVCAR-3 to evaluate migration rate at 5 days, after combinational and monotherapy treatment. Original magnification 4x. Scale bar = 300 µM **(D)**. Representative results for the quantitative measurement of wound closure for same experiment group **(E)**. **Transwell assay.** Images were taken by fluorescence microscopy, original magnification 10X. Scale bar = 10 µM. **The results demonstrate a decrease in invasion capacity in *188-5p* transfected resistant in compared with control group after Olaparib treatment. Results indicated that combinational treatment is effective for decreasing the wound healing capability in resistant cells.**

In the Western blot studies conducted as part of this thesis study, it was revealed that the level of the epithelial marker E-cadherin protein was higher in parental cells compared to resistant cells. This result is consistent with the information in the literature. In contrast to E-cadherin, N-cadherin is a mesenchymal cell marker and operates on the opposite principle. These findings indicate that combination therapy helps reduce the level of N-cadherin protein, thereby inducing mesenchymal-epithelial transition and reducing mesenchymal cell formation in resistant cells **(Figure 3.23).**

N cadherin and E-cadherin are crucial EMT markers. E-cadherin is primarily found in epithelial tissues, where it plays a key role in mediating cell-cell adhesion, while N-cadherin is primarily associated with neural tissues and contributes to neural development and cell migration. Loss or downregulation of E-cadherin is often associated with increased invasiveness and metastasis in various cancers, this phenomenon, known as the "cadherin switch," is a hallmark of the epithelial-to-mesenchymal transition (EMT) during cancer progression. After wound healing assay, we checked the protein levels of N-cadherin and E cadherin of our samples. As expected, resistant cells have higher N-cadherin protein level than their parental and with the combinational therapy of *188-5p* and Olaparib N-cadherin protein level decreased and E-cadherin level is increased in resistant cells, proves that *188-5p* has an anti-metastatic effect. In the meantime, monotherapy of *188-5p* also play role in decreasing the N-cadherin and increasing the E-cadherin level dramatically depend on our western blot results (**Figure 3.23 A-B**).

To enlighten the migration capacity of Olaparib resistant OVCAR-3 cells, we performed the wound healing assay and transwell with *188-5p* transfected resistant cells and compared with negative control cells **(Figure 3.23 C-D-E).** Our results demonstrate that synergistic effects of *188-5p* and Olaparib importantly decreased EMT mechanism of resistant cells and suppress the metastasis capacity.

Table 3.8: Candidate mRNA targets of miR-*188-5p* based on in silico analysis.

Target Score	Gene Symbol	Gene Description	Function
97	FOXN2	forkhead box N2	Supression of EMT
84	XRCC5	X-ray repair cross complementing 5	Double Strand Break repeair
78	LARP4	La ribonucleoprotein domain family member 4	BID Inhibitor
65	CCNG1	cyclin G1	Cell Cycle
65	RAD51D	RAD51 paralog D	Homologous Recombination Mechanism
56	TMBIM1	transmembrane BAX inhibitor motif containing 1	BAX Inhibitor

Depend on in silico-based analysis, we listed potential mRNA targets of miR-*188-5p* as Cyclin G1 (CCNG1), X Ray Repair Cross Complementing 5 (XRCC5), RAD51 parolog D (RAD51D), Transmembrane BAX Inhibitor Motif Containing I (TMBIM1) and La ribonucleoprotein domain family member 4 (LARP4) and Forkhead Box N2(FOXN2) by using TargetScan and miRDB databases. We believe that these target genes play an important role in resensitization mechanism of Olaparib resistant OVCAR-3 cells and act independently or in combination through AKT signal transduction pathway.

Chapter 4:

DISCUSSION AND CONCLUSION

In recent years, PARP inhibitors (PARP-is) have become increasingly integrated into routine clinical practice, utilized as both first- and second-line maintenance therapies for ovarian cancer (O'Malley et al, 2023). While these agents generally provide a favorable initial response, many patients experience poor prognosis or relapse due to the development of resistance to PARP-is. Understanding the mechanisms underlying this resistance is crucial for improving therapeutic strategies and patient outcomes (Xiaoyu Fu et al, 2024).

Primarily, resistance to PARP-is can occur through three primary mechanisms: restoration of HRR, mitigation of replication stress and upregulation of drug efflux pumps. Tumor cells may restore HRR, which is a critical pathway for repairing DNA-DSBs, thereby reinstating the cell's ability to repair DNA and diminishing PARP inhibition (Cordani et al, 2023). Additionally, tumor cells can adapt to reduce replication stress, which is one of the cellular damages induced by PARP-is (Demeny et al, 2021). Furthermore, increased expression of drug efflux pumps can lead to remove PARP-is from cancer cells, reducing their efficacy. These resistance mechanisms highlight the need for novel therapeutic approaches to prevent or overcome resistance. New promising strategies involve the combination of PARP-is together with other targeted therapies to enhance their efficacy or counteract resistance mechanisms. For example, combining PARPis with AKT inhibitors may inhibit pathways that contribute to resistance and sensitize tumor cells to PARP inhibition (Tain et al, 2023).

AKT plays a pivotal role in the PI3K/AKT/mTOR signaling pathway, and its inhibition has been shown to induce HRD in tumor cells. Currently, several AKT inhibitors are under clinical investigation for treating solid tumors. In the context of platinum-resistant ovarian cancer, the combination of the pan-AKT inhibitor afuresertib with carboplatin

and paclitaxel has demonstrated a significant efficacy (He et al, 2021). However, the potential for AKT inhibition to enhance the response of recurrent EOC to PARPi has remained mostly unexplored. The role of AKT in promoting cell survival and proliferation makes it a promising target for combination therapies to enhance the efficacy of existing treatments. The observed synergy between AKT inhibition and traditional chemotherapeutics in platinum-resistant ovarian cancer suggests that similar strategies could be beneficial when combined with PARPis. This approach could sensitize tumor cells to PARP inhibition by further compromising DNA repair mechanisms through induced HRD (Xu et al, 2022). An AKT inhibitor, LAE003 (also known as Uprosertib) has been identified as a potent agent in reducing tumor cell growth in both platinum-resistant and PARP inhibitor-resistant ovarian cancer patients. Notably, when combined with the PARP inhibitor Olaparib, LAE003 exhibited an additive increase in anti-tumor response. The combination of AKT inhibition and PARP inhibition presents a compelling therapeutic strategy for advanced ovarian cancer patients, particularly those who have developed resistance to conventional treatments. The dual inhibition of AKT and PARP pathways represents a promising approach to overcoming drug resistance in ovarian cancer therapy (Xu et al, 2022).

Given the central role of the PI3K/Akt pathway in cancer and drug resistance, targeting this pathway has become a promising therapeutic strategy. Various inhibitors targeting different components of the pathway, including PI3K inhibitors (e.g., alpelisib), Akt inhibitors (e.g., ipatasertib), and mTOR inhibitors (e.g., everolimus), are currently in clinical development or have been approved for use in certain cancers. MicroRNAs (miRNAs) play a crucial role in the post-transcriptional regulation of genes involved in the PI3K/Akt pathway. Dysregulation of specific miRNAs can lead to altered expression of pathway components, further contributing to tumorigenesis and resistance to therapy. For instance, miRNAs that normally suppress oncogenes might be downregulated, activating the PI3K/Akt pathway. An example of this mechanism includes miR-*182-5p* and miR-*205-5p*, which act as negative regulators of Akt. Upregulation of these miRNAs leads to Akt inhibition, slowing down cancer cell proliferation and acting as tumor suppressor genes (Xu et al, 2014; Xu et al, 2018). In contrast, some oncoMIRs, such as miR-*122*, positively regulate the PI3K/Akt pathway and stimulate its activation (Lian et al, 2013).

In EOC, the role of many miRNAs targeting the Akt pathway has been characterized in relation to chemoresistance against conventionally used drugs. Pertuzumab, a humanized monoclonal antibody, is used in ovarian cancer treatment, but the molecular mechanisms underlying pertuzumab's anti-tumor are not fully understood. Studies have shown that Pertuzumab treatment increases miR-*150* levels in OC cells. Inhibition of miR-*150* level triggers the PI3K-Akt pathway, resulting in pertuzumab resistance in OC cells (Wuerkenbieke et al, 2015). Another study investigating the molecular mechanisms of epithelial ovarian cancer resistance found that differential expression of miR-*301*a is related to enhanced drug resistance by triggering PI3K/AKT signaling in EOC (Ni et al, 2018).

Cisplatin resistance in EOC is one of the major reasons for poor prognosis. miR-*7704* was found to be significantly downregulated in cisplatin resistant ovarian cancer cells. Overexpression of miR-*7704* overexpression led to downregulation of IL2RB gene, resulting in inactivation of the AKT signaling pathway and increased cisplatin sensitivity (Meng et al, 2024). Paclitaxel is used in epithelial cancer treatment, often in combination with platinum-based drugs such as cisplatin and carboplatin. In a study focused on the effect of miR-*92a* on paclitaxel resistance in EOC through its interaction with PTEN. Deng and her colleagues were observed that upregulation of miR-*92a* resulted in decreased apoptosis, enhanced resistance to paclitaxel via activation of AKT signaling (Deng et al, 2023).

The principle of synthetic lethality underpins the efficacy of PARPis in cells deficient in HR, such as those with BRCA1/2 mutations and RAD51. Several miRNAs have been found to increase sensitivity to Olaparib when over-expressed. Notably, hsa-miR-*107* and hsa-miR-*222* were identified as key regulators of the DDR. These miRNAs sensitize tumor cells to Olaparib by repressing the expression of RAD51, a crucial protein in the HR repair pathway. Characterizing hsa-miR-*107* and hsa-miR-*222* as key sensitizers to Olaparib offers promising new biomarkers for predicting PARPi response and potential targets for enhancing therapeutic efficiency (Neijenhuis et al. 2013). Additionally, in resistant ovarian cancer cell lines, silencing miR*181-a* sensitized cells to Olaparib, highlighting miR-*181a* as a potential biomarker and therapeutic target in ovarian cancer (Bustos et al, 2023). Also, a research paper focusing on the role of miR-*622* in OC

indicated that Ku complex, a key component of the non-homologous end-joining (NHEJ) pathways, is mRNA target of miR-622. miR-622-mediated restoration of HR contributes to therapeutic resistance (Choi et al, 2016). miR-200c has been identified to target NRP1 in OC cells. Overexpression of miR-*200c* in OC cells resistant to Olaparib has been shown to restore sensitivity to the drug, suggesting that miR-*200c*-mediated downregulation of NRP1 can counteract resistance mechanisms, thereby enhancing the efficacy of Olaparib (Vescarelli et al, 2020)

miR-*188-5p* is known as anti-proliferative, anti- metastatic miRNA in HGSOC and act as tumor suppressor gene during cancer progression. This thesis study investigated the effects of combinational therapy with *188-5p* and Olaparib on resistant cells in HGSOC as *ex vivo* and *in vitro* for the first time.

The aim of this thesis study was to investigate the synergistic role of miR-*188-5p* and Olaparib in resistant HGSOC for the first time; by using *in vitro* cell lines and *ex vivo* patient derives samples. Based on differential expression analysis of miRNAs, 10 candidate miRNAs were selected from resistant FFPE and *in vitro* cell samples (Table 3.2). To determine significant differential expression levels of miRNAs between drug-resistant and drug-sensitive cells and to validate microarray results in FFPE patient tissues, qRT-PCR studies were successfully completed on a total of 40 FFPE patient samples and *in vitro* resistant and parental cells (Figure 3.5 and 3.6). As a result, we showed that miR-*188-5p* was shown to be significantly downregulated in resistant HGSOC. The results showed that the synergistic combination of miR-*188-5p* mimic and Olaparib decreased cell proliferation and viability and induced apoptosis in resistant cells (Figure 3.8, 3.9 and 3.10). A major result of the thesis study was the observation of a significant decrease in the level of phospho-AKT in the combinationally treated resistant cells (Figure 3.18). These results support literature findings related to the suppression of Akt and anti-survival mechanisms of drug resistance by regulating double strand break mechanisms (Graphical abstract of the results were shown in Figure 4.1).

DSBs represent a severe form of chromosomal damage that can precipitate genomic instability, potentially culminating in carcinogenesis. The histone variant H2AX undergoes rapid phosphorylation at serine 139 by members of the phosphoinositide 3-kinase family of enzymes in response to DNA double strand breaks (Podhorecka, 2010).

Phosphorylated histone H2AX serves as a sensitive indicator in DSBs, contributing to genomic instability and influencing cancer treatment. Thus, investigating the relation between levels of phosphorylated H2AX and miRNA regulation may provide a sensitive approach to tracking cancer progression and treatment response (Suzuki et al, 2023). For instance, upregulation of miR-134 resulted in increased H2AX S139 phosphorylation, promoting DNA repair mechanism through non-homologous end joining (NHEJ), and reducing chemosensitivity in ovarian cancer cells (Wu et al, 2016). In response to DNA damage or replication stress, the ATM and ATR checkpoint pathways are triggered to inhibit cell cycle progression. This allows time for the cell to repair DNA lesions and resolve replication stress (Maréchal et al, 2013). CHK1, a key downstream effector of the ATR checkpoint pathway, serves as a critical regulator of the cellular response to both DNA damage and replication stress. Dysregulation of CHK1 can lead to detrimental outcomes such as cell death and genomic instability. Phosphorylation of CHK1 by AKT at serine 280 leads to the localization of CHK1 in the cytoplasm, which restricts its functions on activation of ATR induced DDR mechanism (Gralewska et al, 2020; Tonic et al, 2010).

There is an inverse relationship between the activity of serine/threonine kinase Akt and H2A.X phosphorylated at Ser16 (H2A.X S16ph). Akt phosphorylation at Ser19 results in inhibition of H2AX phosphorylation, promoting cancer cell survival. This Akt-mediated phosphorylation of H2AX offers new insights into cancer pathogenesis and treatment. In our results, we observed significant upregulation of H2AX protein levels by western blot and immunofluorescence experiments (Figure 3.19). Literature suggests that decreased levels of p-AKT and upregulation of p-H2AX induce cancer cell death mechanisms. To investigate the apoptosis mechanism, we performed Annexin-V PI staining with flow cytometry and IF experiments and several apoptosis targets were investigated at gene and protein levels by western blot and q-PCR experiments. These results showed a significantly increased apoptosis level upon synergistic treatment with Olaparib and miR-*188-5p* in resistant HGSOC cells. Apoptosis induction in resistance is a primary mechanism by combinational treatment exerts cytotoxic effects. However, the PI3K/Akt pathway can counteract apoptosis through the phosphorylation and inactivation of pro-apoptotic factors like BAX, and the activation of anti-apoptotic proteins such as Bcl-2 (Datta et al., 1999). This anti-apoptotic signaling is a key contributor to therapeutic

resistance, as tumor cells with hyperactive PI3K/Akt signaling are less likely to undergo apoptosis in response to treatment.

The activated phosphorylated AKT detaches from the cell membrane and moves through the cytoplasm or to the cell nucleus, where it can activate or inhibit downstream apoptotic protein molecules, such as Bim, Bid, Bad, BAX, caspase 9, and forkhead transcription factor family. In the apoptosis mechanism, caspases drive apoptosis through the activation of pro-apoptotic initiator molecules such as Bim and Bid. Initiator caspases (e.g., caspases-8 and -9) activate effector caspases (e.g., caspases-3 and -7), which cleave structural proteins, leading to cell death (Jeong et al, 2008). Caspase-3 cleaves the DNA repair enzyme PARP, making cleaved-caspase-3 and cleaved-PARP crucial regulators of apoptosis (Los et al, 2002). It is very important to target these genes, especially for ovarian cancer, which has DNA damage breaks, such as HGSOC (Reid A. et al, 2017) In our study, combinational therapy with *188-5p* and Olaparib has been shown to promote apoptosis and enhance tumor cell sensitivity, as shown by Western blotting, Flow Cytometry and IF analysis (Figure 3.13, 3.14 and 3.16). Combinational therapy activates caspase cascades and increases cleaved-caspase-3 and cleaved-PARP expression, confirming its role in promoting apoptosis in ovarian cancer (Figure 3.17).

In extrinsic apoptosis mechanism, upon binding tumor necrosis factor-related apoptosis-inducing ligand to its receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2), TRAIL forms the death-inducing signaling complex (DISC) by recruiting Fas-associated death domain, which in turn recruits and activates caspases-8/10. Activated caspase-8 can then directly cleave downstream effector caspases (caspase-3, -6, -7), leading to extrinsic mediated apoptosis, or cleave Bid to its pro-apoptotic form (tBid), which activates the intrinsic apoptosis pathway by targeting the mitochondrial membrane (Goncharenko-Khaider et al, 2010). In our results, significantly activated caspase-8 levels triggered extrinsic apoptosis mechanisms, and elevated levels of Bid mRNA expression were concluded as regulators of mitochondrial- intrinsic apoptosis mechanism upon treatment with *188-5p* mimic and Olaparib in HGSOC cells (Figure 3.21 and 3.22). In synergistically treated Olaparib Resistant OVCAR-3 cells, Akt suppression increased the Bid and Bim protein levels and active Bid protein interferes with the mitochondrial activation of apoptosis.

Thus, mitochondrial activation stimulates the feedback loop required to fully activate caspase-8 in the cytosol.

Akt-induced epithelial-to-mesenchymal transition (EMT) involves the downregulation of E-cadherin due to the upregulation of the transcription repressor Snail. E-cadherin is one of the molecules that provides intercellular adherent bonds and is responsible for the acquisition of epithelial phenotype and determination of cell shape and polarity (Loh et.al, 2019). E-cadherin is also involved in intracellular signaling pathways such as PI3K/AKT pathway (Downward et al., 2004). Studies in the literature show that activation of the PI3K/Akt axis emerges as a central feature of EMT. EMT induced by Akt induces downregulation of E-cadherin resulting from upregulation of the transcription repressor SNAIL (Hong KO. et al. 2009). In our results, resistant ovarian cancer cells may undergo mesenchymal-to-epithelial reverting transition (MErT), with E-cadherin re-expression being a key indicator. Our study investigated whether Akt inhibition could restore E-cadherin expression, induce MErT upon synergistic treatment with 188-5p mimic and Olaparib in resistant HGSOC cells.

N-cadherin is a mesenchymal cell marker and has the opposite working principle to E-cadherin (Issagholian et al., 2023). One of the most important findings of this study is the significant decrease in N-cadherin levels in resistant cells given the mimic and Olaparib combination. In line with the results obtained, with the inhibition of the AKT pathway caused by the decreased AKT phosphorylation, the EMT mechanism is reversed, resulting in a decrease in SNAIL and N-cadherin protein levels and an increase in E-cadherin levels (Figure 23 A-B). This finding revealed that the combination therapy induced mesenchymal-epithelial transformation and helped to reduce mesenchymal cell formation in resistant cells. Furthermore, transwell assay and wound healing assay results demonstrate that synergistic interaction significantly suppresses the metastatic capacity of cells (Figure 3.23 C-E)). These findings demonstrate that the use of combinational therapy with miRNA mimics and drugs in HGSOC cell treatment has high potential to overcome drug resistance and is compatible with the hypothesis of this thesis project.

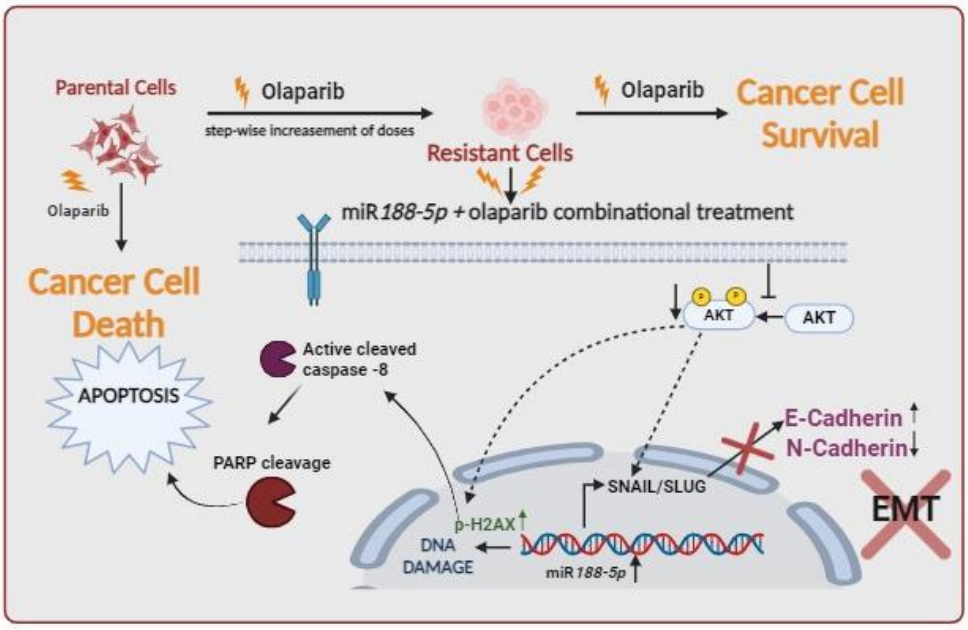


Figure 4.1: Graphical abstract of the study (created by biorender).

FUTURE ASPECTS

In this thesis study, potential mRNA targets of miR-*188-5p* were indicated as Cyclin G1 (CCNG1), X Ray Repair Cross Complementing 5 (XRCC5), RAD51 parolog D (RAD51D), Transmembrane BAX Inhibitor Motif Containing I (TMBIM1) and La ribonucleoprotein domain family member 4 (LARP4) and Forkhead Box N2(FOXN2) due to in silico-based analysis by using TargetScan and miRDB databases (Table 3.8). We believe that these target genes play an important role in resensitization mechanism of Olaparib resistant OVCAR-3 cells and act independently or in combination through AKT signal transduction pathway.

G1 cyclins are responsible proteins for G1-S and G2 cell cycle check point control in cells. They act as oncogenes by stimulating progression and metastasis of ovarian cancer (Bertoli et al, 2013). In our study, we showed that combinational therapy with *188-5p* and Olaparib induce subG1 arrest in resistant cell lines and cell proliferation and metastasis levels were suppressed. In literature, CCNG1 gene was found related with miR-23-b which is a key regulator miRNA in proliferation and metastasis in ovarian cancer. In this research study, miR-23-b was found significantly downregulated in ovarian cancer cells and by upregulating miR23-b level, CCNG1 gene was suppressed, thus ovarian cancer cell proliferation, invasion, and migration was inhibited, and apoptosis was induced (Yan et al, 2016). We believe that 188-5p/CCNG1 relation could show important effect on induced apoptosis levels in resistant HGSOC.

XRCC5(Ku80), forms a DNA-dependent protein kinase complex with XRCC6. Dysfunction of XRCC5 can lead to chromosomal instability, promoting tumorigenesis. XRCC5 is implicated in tumor development and acts as an adhesion factor in tumor cell adhesion, invasion, and migration in many cancer types including breast and ovarian and renal cancer. For instance, down-regulation of XRCC5 promotes apoptosis in renal cancer cells, suggesting its role in renal cancer progression by inhibiting apoptosis. Similarly, down-regulation in esophageal squamous cell carcinoma inhibits tumor cell proliferation. In breast cancer cells, reduced XRCC5 expression enhances invasion and migration by inhibiting tropomyosin receptor kinase A. The regulatory effects of XRCC5 and miR*188-5p* were shown in glioma and Natural killer/T cell lymphoma (NKTL). Upregulation of

XRCC5 due to low level of *188-5p* in glioma and NKTL cells, was identified as key component in tumor and metastatic progression of these diseases (Leng et al, 2021; Huang et al, 2021).

RAD51D gene, encoded by the RAD51 protein family, have crucial role in by HR pathway by facilitating DSB repair mechanism. During DSB repair mechanism in resistance, RAD51 protein family involved the DSB and reformed genomic instability (Reh et al, 2017). Two miRNAs, miR-103 and miR-107 were fond associated with RAD51 mediated re-sensitization. These miRNAs inhibited RAD51 foci formation, reduced homology-directed repair, and sensitized cells to DNA-damaging agents such as cisplatin and PARP inhibitors in ovarian cancer (Huang et al, 2013). Depend on this thesis results, elevated level of double strand break elements such as p-H2AX we suggested that XRCC5/188-5p interactions could be promising strategy for overcome drug resistance in HGSOC.

TMBIM1, a BAX inhibitor gene, have an active role to regulate extrinsic apoptosis pathway by negatively affecting receptor binding activity and Fas mediated signaling pathway (Liu et al, 2017). As shown in this thesis results about the effects of combinational therapy with *188-5p* and Olaparib on apoptosis, we concluded that TNF receptor family gene levels are upregulated, and caspase-8 mediated extrinsic apoptosis are a triggered. Upregulation of miR-*188-5p* could regulate TMBIM1 gene level as a downstream target gene. By inhibiting TMBIM1 gene extrinsic apoptosis could be altered as indicated in this thesis findings.

LARP4 is a member of the LA related proteins and found strongly related with cancer cell migration and proliferation in many cancer types including epithelial ovarian cancer, breast cancer and glioma (Jeniffer et al 2023). La protein family have a crucial role in anti-apoptotic BCL-2 family members related with cell survival mechanism in epithelial ovarian cancer. In addition to this, La proteins found associated with PI3K/AKT pathway by regulating beta catenin which is a substrate of AKT signaling (Huang et al, 2020). Downregulation of LARP4 gene upon increasement in miR*188-5p* expression in resistant HGSOC, may trigger the *188-5p*/ AKT/LARP4 axis to induce apoptosis.

In the literature, FOXN-2 gene was confirmed as mRNA target of miR*188-5p* and induce anti-proliferative effect on epithelial ovarian cancer (Wang et al, 2024). However, the function of *188-5p*/FOXN2 axis on resistant ovarian cells remain unclear, we aim to explore effect of FOXN2 gene on re-sensitization mechanism in HGSOC. On the other hand, there is not any investigations in the literature about relation between Cyclin G1, XRCC5, RAD51D, TMBIM1 and LARP4 target genes and *miR188-5p* in ovarian cancer. mRNA target genes which characterized as possible regulators of 188-5p/AKT axis will be determined in protein levels by western blot analysis and mRNA-miRNA relation will be observed with Luciferase Reporter assay.

As a second future aspect of this thesis study, combinational therapies could be expanded to investigate other potential combinational treatments involving *188-5p* mimics with different PARP inhibitors or other chemotherapeutic agents to identify synergistic effects and broaden treatment options for resistant ovarian cancer. Also *in vitro* cell line studies could be enlarged to investigate whether the combination of *188-5p* mimics and PARP inhibitors could be effective in other cancer types where PARP inhibitor resistance is an issue, potentially expanding the clinical applications of this approach.

As third, to identify miR-*188-5p* as universal biomarker for cancer resistance, *188-5p* synergy with PARPis should be conducted to large scale patient cohort for confirming its prognostic value and potential use in clinical settings in HGSOC. Therefore, by using *in vivo* preclinical models to study the effects of *188-5p* upregulation in combination with Olaparib will be improved the knowledge on the tumor growth inhibition, survival benefits, and potential resistance mechanisms. In the future, clinical trials could be improved with Phase I/II Trials to test the combination of *188-5p* mimics and Olaparib in ovarian cancer patients with Olaparib-resistant tumors and design clinical trials that stratify patients based on their *188-5p* expression levels to determine the most effective treatment strategies for different patient subgroups. By pursuing these future research directions, we can deepen our understanding of *188-5p* role in Olaparib resistance and develop innovative therapeutic strategies to improve outcomes for patients with resistant ovarian cancer.

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APPENDIX-A:

CURRICULUM VITAE

EDUCATION

	DEPARTMENT	INSTITUTION	DEGREE
2020- 2024	Hücresel ve Moleküler Tıp	Koç Üniversitesi	Doctoral
2016-2018	Moleküler Biyoloji Genetik ve Biyoteknoloji	İstanbul Teknik Üniversitesi	Master
2011-2015	Moleküler Biyoloji ve Genetik	Yeni Yüzyıl Üniversitesi	Undergraduate

ACADEMİC EXPERIENCES

	POSITION	DEPARTMENT	ENSTITUTION
2020-2024	Teaching Assistant/ PhD Researcher	Hücresel ve Moleküler Tıp	Koç Üniversitesi
2019-2020	Researcher/PhD	Tıbbı Biyoloji	Acıbadem Üniversitesi
2016-2018	Master Student/ Teaching Assistant	Moleküler Biyoloji ve Genetik	İstanbul Teknik Üniversitesi

2014-2015	Undergraduate Student/ Intern Researcher	Moleküler Biyoloji ve Genetik	Yeni Yüzyıl Üniversitesi

SEMINARS and CONFERENCES

2023	European Congress on Gynaecological Oncology (ESGO 2023	İstanbul, Türkiye
2023	Annual Congress of European Association for Cancer Research (EACR23)	Torino, Italy
2021	6th European Congress of Immunology (ECI2021)	Virtual Event, Worldwide
2019	1st International Multidisciplinary Cancer Research Congress (MOKAD 2019)	Diyarbakır, Türkiye
2018	Genome Maintenance, DNA Repair and Cancer	Acibadem University
2017	5th International Congrees of Molecular Biology Association of Turkey	Boğaziçi University
2012	Multidisciplinary R&D and Innovation on Life Sciences Symposium	Istanbul University

PUBLICATIONS

2023	Investigation of combination treatment with mirnas and conventional drugs synergistically in resistant HGSOC cells. DOI: 10.1136/ijgc-2023-ESGO.775
2023	Investigating the role of MiRNAs in ovarian cancer stem cells and their relation to drug resistance. DOI: 10.1136/ijgc-2023-ESGO.896
2023	Targeting Ovarian Cancer with Chalcone Derivatives: Cytotoxicity and Apoptosis Induction in HGSOC Cells. DOI: 10.3390/molecules28237777
2023	Tetraphenylethylene-Based Photoluminescent Self-Assembled Nanoparticles: Preparation and Biological Evaluation DOI: 10.1021/acsmedchemlett.3c00396
2022	Development of Dual and Single PCR Protocols for Intestinal Helminths: A Preliminary Study on Multiplex PCR. DOI: DOI: 10.54453/TMCD.2022.47135

2021	Expression of the B-cell stimulatory factors BAFF and APRIL in gastric precancerous lesions and gastric cancer. WOS:000753366401561
2021	Influence of *H.pylori* density on PD-1:PD-L1 expression in gastric precancerous malignancies. WOS:000753366401562
2021	The crosstalk between H. pylori virulence factors and the PD1:PD-L1 immune checkpoint inhibitors in progression to gastric cancer. DOI: 10.1016/j.imlet.2021.06.009.
2021	Characterisation of the Leishmania donovani/L. infantum Hybrid Isolated from an Autochothonous Kala-Azar Patient: Preliminary Results of an *In Vivo* Model. DOI: 10.4274/tpd.galenos.2021.14622.
2018	Alterations of growth rate and gene expression levels of UPEC by antibiotics at sub-MIC. DOI: doi: 10.1007/s12223-017-0582-z. Epub 2018 Jan 11.

AWARDS

- Koç University Graduate School of Health Sciences, Academic Excellence Award (2024)

INTERESTS

- Molecular Oncology and Cancer Research
- Ovarian Cancer
- Drug Resistance Mechanisms
- Immunology