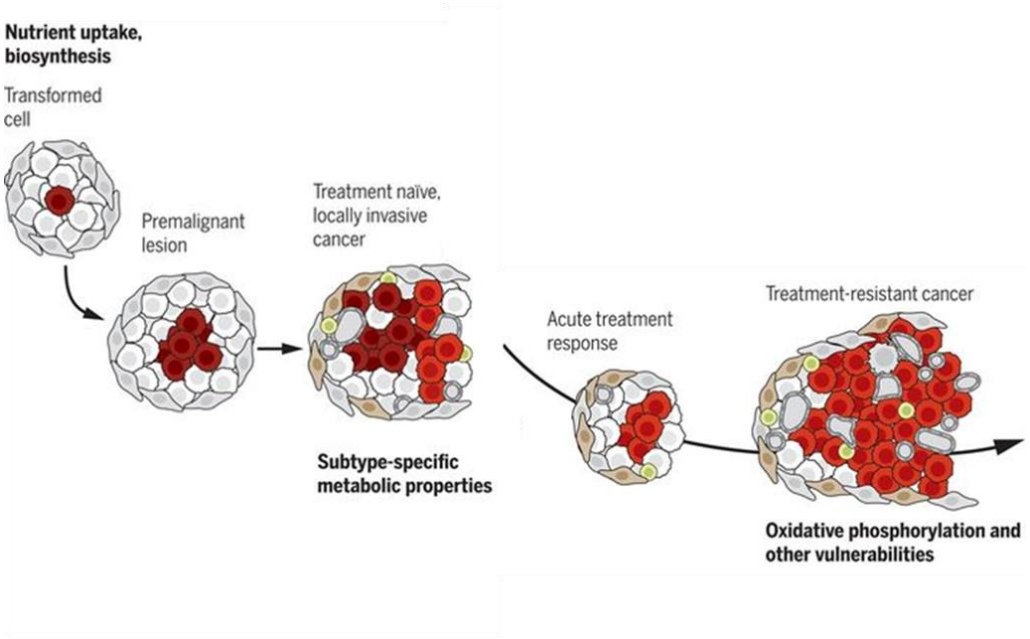
UNIVERSITY OF NAPLES FEDERICO II

DOCTORATE IN
MOLECULAR MEDICINE AND MEDICAL BIOTECHNOLOGY

XXXIV CYCLE

Daniela Criscuolo

Role of metabolic reprogramming in the development of ovarian cancer platinum resistance



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Role of metabolic reprogramming in the development of ovarian cancer platinum resistance

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LIST OF ABBREVIATIONS

ACLY: ATP-citrate lyase

BSO: buthionine sulfoximine

CAV-1: caveolin -1

CTR: Copper transporter

DHCR24: 24-Dehydrocholesterol reductase

EOC: Epithelial ovarian cancer

FDFT1: Farnesyl-diphosphate farnesyltransferase 1

FDPS: Farnesyl diphosphate synthase

GCLC: glutamate-cysteine ligase catalytic subunit

GSH: Reduced glutathione

GSR: glutathione disulfide reductase

GSS: glutathione synthase

GST: Glutathione S-transferase

HGSOC: High grade serous ovarian cancer

HMGCR: 3-Hydroxy-3-methylglutaryl-CoA reductase

HMGCS1: 3-Hydroxy-3-methylglutaryl-CoA synthase 1

HR: Homologous recombination

LDLR: Low-density lipoprotein receptor

LIPG: Lipase G, endothelial type

MMR: mismatch repair

MSMO1: Methylsterol monooxygenase 1

NADPH: Nicotinamide adenine dinucleotide phosphate

NER: nucleotide excision repair

NPC1: Niemann-Pick C1 protein

OC: Ovarian cancer

OSC: 2,3-Oxidosqualene cyclases

OXPHOS: Oxidative phosphorylation

PARP: Poly (ADP-ribose) polymerase

ROS: Reactive oxygen species

SQLE: Squalene Epoxidase

TCA: Tricarboxylic acid

TRAP1: Tumour necrosis factor receptor-associated protein 1

TRXRD1: thioredoxin reductase 1

γ-GCS: γ-glutamilcysteine synthetase

1. INTRODUCTION

1.1 Ovarian cancer

1.1.1 Epidemiology, risk factors and pathologic classification

Ovarian cancer (OC) is the most aggressive gynecological malignancy and the fifth leading cause of death among women (1). According to the latest Global Cancer Observatory (GLOBOCAN) report, OC accounted for 1.6% of all cancers and 2.1% of all cancer deaths worldwide in 2020 (2). A higher incidence of OC has been estimated in the United States, Canada, Eastern and Centre Europe (>8 per 100,000 population) than in Africa and Asia (<4 per 100,000 population) (2). Multiple factors have been associated with the risk of developing OC, including genetic susceptibility, age, hormonal therapy use, nulliparity and infertility, environmental and lifestyle factors (3). Among these, family history of ovarian and/or breast cancer is the most important risk factor: approximately 25% of all OC cases are associated with inherited conditions (4). Susceptibility genes for OC are mainly involved in homologous recombination and mismatch repair pathways, primarily BRCA1/2 and MSH1/6 (4). It has been estimated that germline pathogenic mutations in BRCA1 or BRCA2 gene increase the lifetime risk of developing OC by 40-60% and 10-30%, respectively (4). Ovarian cancer is a heterogenous disease which encompasses a group of neoplasms with distinct cells of origin, risk factors, clinical and molecular features and prognosis. According to the World Health Organization's classification system, ovarian tumors are subdivided into three main categories based on cells of origin: epithelial cancers, derived from surface cells of the ovary, peritoneum or fallopian tubes; sex cord stromal tumors, derived from stromal cells (fibroblasts, theca and Leydig cells) and primitive sex chord cells (granulosa and Sertoli cells); germ cell tumors, derived from the ovum producing cells of the ovary (Fig. 1A). The most common subtypes, epithelial ovarian cancers (EOCs) account for 90% of ovarian tumors. There are five main hystotypes of EOCs: high-grade serous (70%), endometrioid (10%), clear cell (10%), mucinous (3%), and low-grade serous (<5%). Moreover, EOCs have been further classified as type I or type II based on morphological and molecular features (Fig. 1B) (5). Type I cancers are low grade of serous, mucinous, endometrioid and clear cell histotypes, that grow slowly, are often diagnosed at an early stage (I or II) and may respond to hormone therapy (6). Type II cancers are high grade serous, high-grade endometrioid and undifferentiated carcinomas, that grow fast, are mostly diagnosed at stage III or IV and respond to conventional chemotherapy (6). The predominant subtype, HGSOC, is commonly diagnosed at advanced stages (III or IV) when the disease is disseminated (7). Due to the absence of an anatomical barrier, HGSOC has a remarkable tendency to early disseminate within the abdominal cavity where the exfoliated cancer cells are transported by peritoneal fluid (8). Moreover, about 80% of patients relapse and develop chemoresistant disease (9). Therefore,

HGSOC is characterized by an exceptionally poor prognosis with five years survival rate of ~43% (10).

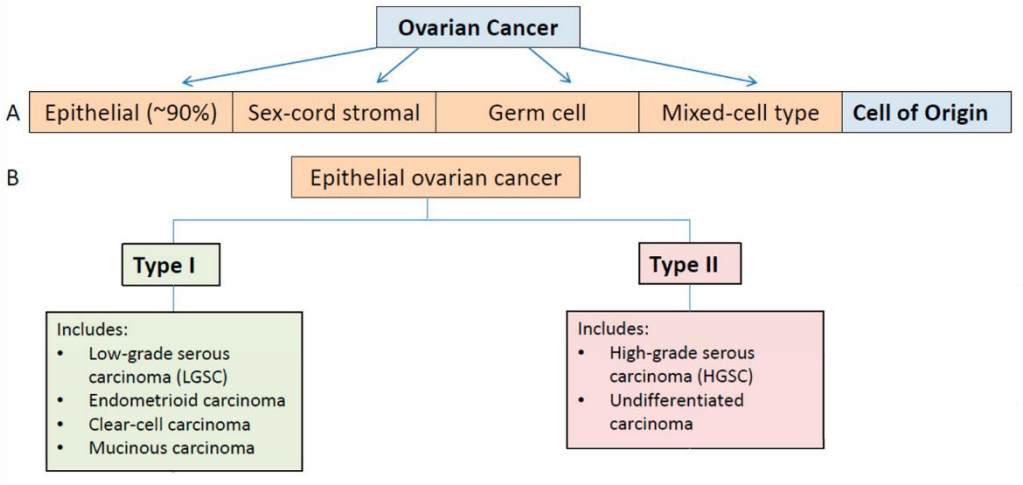

Figure 1. ***(a)*** *Histological subtypes of ovarian cancer and (**b**) widely accepted epithelial ovarian cancer classification paradigm based on clinicopathologic and molecular evidence (11).*

1.1.2 Therapeutic options

The standard management strategy for OC consists of platinum-based chemotherapy before or after debulking surgery. In particular, the initial chemotherapy regimen involves the combination of platinum agents (cisplatin or carboplatin) and taxanes (paclitaxel or docetaxel). Although approximately 80% of patients respond well to the initial treatment, a high rate of recurrence has been observed (12). Based on the platinum-free interval, defined as the time elapsed between the last cycle of chemotherapy and relapse, the patients can be classified as platinum-sensitive, platinum-resistant and platinum-refractory (13). Patients who relapse at least 6 months after the completion of chemotherapy are considered platinum-sensitive, while if the relapse occurs before 6 months they are classified as platinum-resistant. On the other hand, platinum-refractory patients show progression during platinum treatment. The longer platinum-free interval, the higher the likelihood that the patient will exhibit platinum-sensitive disease and respond positively to retreatment with platinum-based chemotherapy (14). Therefore, recurrent platinum-sensitive patients continue to receive a platinum-based therapy, however, usually, the response rate and the treatment-free interval decrease progressively and ultimately the disease becomes platinum-resistant (15). For patients with resistant or refractory disease, non-platinum drugs, including paclitaxel, gemcitabine, topotecan, pegylated liposomal doxorubicin and trabectedin, represent a viable alternative (16). Moreover, other therapeutic strategies have been investigated to improve clinical outcome in OC, such as targeted therapy, immunotherapy, hormone therapy and

radiotherapy (17). Among the targeted therapies, antiangiogenic drugs and poly(ADP-ribose) polymerase (PARP) inhibitors have been the most studied for the treatment of patients with advanced OC. Recently, several clinical trials have shown that the use of PARP inhibitors (olaparib, niraparib and veliparib), as first-line or maintenance therapy in patients with BRCA-associated HGSOC, significantly improves the progression-free survival by delaying relapses (NCT01844986; NCT02477644; NCT02655016) (18). Currently, bevacizumab is the only antiangiogenic compound approved for women with newly diagnosed and recurrent disease in combination with standard chemotherapy (19). Despite many therapeutic options proposed for the initial or recurrent setting, the long-term survival for OC patients remains poor mainly due to the advanced stage at diagnosis and the onset of acquired resistance to platinum-based drugs.

1.2 Platinum-based chemotherapy

1.1.2 Cisplatin mechanisms of action

Cisplatin (cis-diamine-dichloro-platinum II) is a neutral platinum coordination compound composed of a platinum atom surrounded by two chlorine atoms and two ammonia groups (20). Although passive diffusion across plasma membrane is considered the main process responsible for cellular cisplatin uptake , it can also be actively transported into the cell by copper transporters (CTR1/2) (21). Upon entering cells, cisplatin is activated by a series of aquation reactions in which one or both cis-chloro ligands are replaced with water molecules resulting in positively charged species that are highly reactive (22). In the intracellular environment, cisplatin aquation occurs spontaneously due to the relatively low chloride concentration (~2–10 mM) compared to the extracellular space (~ 100 mM) (22). The antitumor activity of cisplatin was initially attributed to its ability to bind nuclear DNA, generating protein-DNA complexes and DNA adducts, which interfere with transcription and replication processes leading to massive cell death (20). Nowadays this view appears as an oversimplification, considering that (i) only ~1% of intracellular cisplatin interacts with nuclear DNA, and (ii) cisplatin have cytotoxic effects even in enucleated cells (20);(23). It is now clear that cisplatin exerts a cytoplasmic toxicity by binding various intracellular molecules, such as cytoskeletal microfilaments, RNAs, thiol-containing proteins and peptides, and thus depleting cells of crucial functions (20). The most important cytoplasmic target of cisplatin is the reduced glutathione (GSH), an essential cellular antioxidant (24);(25). The binding of cisplatin to GSH alters the redox balance leading to the accumulation of reactive oxygen species (ROS) which exacerbate DNA damage and can directly induce apoptosis by opening the mitochondrial permeability transition pore (24). The specific molecular mechanisms by which cisplatin exerts anticancer effects have not yet been elucidated. In particular, the relative contribution of cytoplasmic and nuclear events induced by cisplatin on its cytotoxic activity remains unclear.

1.2.2 Mechanisms of platinum resistance in EOC

The molecular mechanisms contributing to the development of cisplatin-resistance in cancer cells have been classified in four groups based on the alterations (i) in processes preceding the binding of cisplatin to DNA (pre-targeting resistance), (ii) directly related to cisplatin-mediated DNA damage (on-target resistance), (iii) concerning the cell death pathways elicited by DNA damage (post-target resistance), (iv) affecting pro-survival signaling cascades that are not directly activated by cisplatin (off-target resistance) (26).

Pre-target resistance

Cisplatin resistance can result from a reduced intracellular accumulation of reactive drug mainly due to decreased influx, increased efflux or drug deactivation. The copper transporter CTR1 mediates the uptake of a significant fraction of cisplatin, while Cu-ATPase ATP7B, responsible for copper extrusion, is involved in its efflux (21);(27). In line with these observations, exposure to cisplatin reduces CTR1 expression in OC cell lines in a concentration- and time-dependent manner, whereas overexpression of ATP7B correlates with cisplatin resistance *in vitro* and is associated with poor prognosis in OC patients (28);(29). Other plasma membrane transporters have been associated with cisplatin resistance in OC cells by increasing cisplatin export, including the ATP-binding cassette (ABC) transporters such as MRP1 and MRP4 (30). The aqua species of cisplatin have a high reactivity for cysteine-rich protein, especially for reduced GSH (24). The binding of cisplatin to GSH has a dual role, on one hand it partially mediates the cytoplasmic toxicity of cisplatin by increasing the oxidative stress, on the other it reduces the amount of reactive cisplatin molecules (24);(31). Three mechanisms have been proposed to explain the role of GSH in regulating sensitivity to cisplatin. The first mechanism involves the ABC transporter-mediated cisplatin efflux. Cisplatin is conjugated to GSH by glutathione S-transferases (GSTs) and the cisplatin-GSH complexes are extruded from the cell by MRP1 or MRP2 reducing cisplatin intracellular levels (31). The second mechanism involves the rate-limiting enzyme for GSH synthesis, γ-glutamilcysteine synthetase (γ-GCS). Indeed, several cancer cells overexpress GSC to increase the synthesis of GSH. In this way, the more GSH is present within the cells, the more platinum molecules are inactivated (31). The third mechanism involves the copper transporter CTR1. Since GSH can spontaneously bind to copper ions, high levels of GSH deplete the available intracellular copper pool leading to upregulation of CTR1, which is responsible for the uptake of cisplatin as well as copper (32);(33). Thus, elevated levels of GSH can increase the intracellular levels of cisplatin, contrary to the commonly accepted view of GSH as mediator of cisplatin resistance. Therefore, although elevated levels of GSH have been commonly associated with cisplatin resistance in several tumor contexts, including OC, its role in chemoresistance remains controversial (34).

On-target resistance

The cytotoxic effects of cisplatin may be limited by alterations in DNA repair pathways which ultimately lead to the generation of pro-apoptotic signals. The cisplatin-induced DNA lesions are mainly repaired by the nucleotide excision repair (NER) system (35). Consequently, increased NER proficiency has been associated with cisplatin resistance in several preclinical models as well as in cancer patients (36). Among the different proteins contributing to the NER system, ERCC1 has been proposed as a molecular predictor of clinical resistance to platinum-based chemotherapy in EOC (37). However, no significant correlation was found between ERCC1 expression and progression-free survival or overall survival rate in OC patients (38). Platinum-DNA adducts can lead to the generation of post-replicative misincorporation (39). The mismatched bases are recognized by mismatch repair (MMR) system, which attempts to repair the lesion, fails, and triggers DNA damage signaling to induce cell cycle arrest and apoptosis (39). When MMR system is defective, cells can continue to proliferate despite the DNA damage, with accumulation of mismatched nucleotides resulting in a condition known as microsatellite instability. A significant reduction in the expression of MMR genes has been found in OC patients after cisplatin treatment and has been associated with the acquisition of chemoresistance (40);(41). Finally, cisplatin adducts can lead to double-strand breaks that are normally repaired by the homologous recombination (HR) system (42). The presence of HR pathway deficiency results in irreparable DNA damage which leads to cell death. Therefore, HR-deficient tumors are generally more responsive to platinum compounds that their HR-proficient couterparts (43). Defects in HR repair, mainly due to mutations in BRCA1 or BRCA2 genes, have been found in up to 50% of EOC (44). However, secondary intragenic mutations can restore the BRCA1/2 protein functionality and re-establish HR, inducing cisplatin-resistance in initially platinum-sensitive ovarian cancers (44).

Post-target resistance

Platinum resistance can result from alterations in signaling pathways that normally trigger apoptosis or in cell death machinery. The most common alteration involved in cisplatin-resistance is the inactivation of the tumor suppressor gene TP53 that occurs in approximately 50% of all human cancer (45). TP53 protein product, p53, is a transcription factor, whose main function is to maintain genetic stability by inducing cell cycle arrest, DNA repair and possibly apoptosis in response to genotoxic stress (45). Mutations of TP53 has been found in 50-80% of OCs correlated with chemoresistance (46). However, the presence of wild type TP53 is not necessary associated with platinum sensitivity and good prognosis (47). Apoptosis is regulated by pro- and anti-apoptotic proteins, mainly factors of the Bcl-2 family, which regulate the release of cytochrome c from mitochondria, and IAP family, that inhibit apoptosis interacting with caspases, the main executioners of the apoptotic machinery (48). Aberrant expression levels of anti-apoptotic factors of Bcl-2 family, including

Bcl-2, Bcl-XL and Mcl-1, and IAP family, such as XIAP, cIAP1/1 and survivin, have been found in OC patients (49);(50). Indeed, small molecules able to inhibit the activity of these factors, have been proposed as strategy to overcome OC cells resistance to chemotherapy (49);(50).

Off-target resistance

Several evidence suggests that alterations in pathways not directly activated by cisplatin, but able to counteract the cisplatin-induced lethal effects, are involved in the development of chemoresistance. A relevant example is constituted by autophagy, an intracellular degradative process aimed at recycling proteins and cytoplasmic components in response to stress conditions components (51). Autophagy shows a dual role in cancer physiology. It can play a tumor-suppressive role avoiding the accumulation of damaged organelles and proteins that could be a source of genomic instability and thus promote tumorigenesis. Nonetheless, autophagy may act as an oncogenic mechanism by providing biomolecules to support the metabolic needs of cancer cell (52). Overall, autophagy has an oncogenic role in the early stages of cancer development and a suppressive role in the advanced stages. During chemotherapy, autophagy can initially be activated as a protective strategy to escape apoptosis by sequestering and degrading the damaged organelles (53). Autophagy induction has been correlated with acquired cisplatin resistance in OC cells, in which cisplatin treatment induces autophagy activation, while inhibition of autophagy promotes cisplatin-induced apoptosis (54). Therefore, autophagy targeting has been proposed as a strategy to increase the effects of chemotherapy and circumvent drug resistance.

1.3 Metabolic rewiring as mechanism of platinum resistance in ovarian cancer

1.3.1 Role of metabolic remodelling in cancer progression

Metabolic reprogramming refers to the ability of cancer cells to modify their metabolic activities to support the acquisition and maintenance of malignant properties. Since altered metabolism has been widely observed across many types of cancer cells, rewired metabolism is considered a hallmark of cancer (55); (56). Cancer cell metabolism is affected directly or indirectly by genetic and epigenetic backgrounds as well as tumor microenvironment. These intrinsic and extrinsic mechanisms converge to alter the metabolic pathways involved in energy production, macromolecular synthesis and redox balance, in order to meet the bioenergetic and biosynthetic needs of proliferating cancer cells and counteract the oxidative stress (57). The metabolic phenotype evolves during cancer progression, from premalignant lesions to overt cancer, enabling cancer cells to optimize their fitness (58). In early stages of tumorigenesis, cancer cells require nutrient uptake and biosynthesis in order to sustain tumor growth. In later stages, cancer cells have to face various metabolic challenges, including

proliferating in a nutrient-altered and hypoxic microenvironment, colonizing distant sites from the primary tumor and surviving exposure to anticancer drugs. These conditions impose different metabolic requirements (58). An overview of the main metabolic pathway rewired in cancer cells is shown in Figure 2.

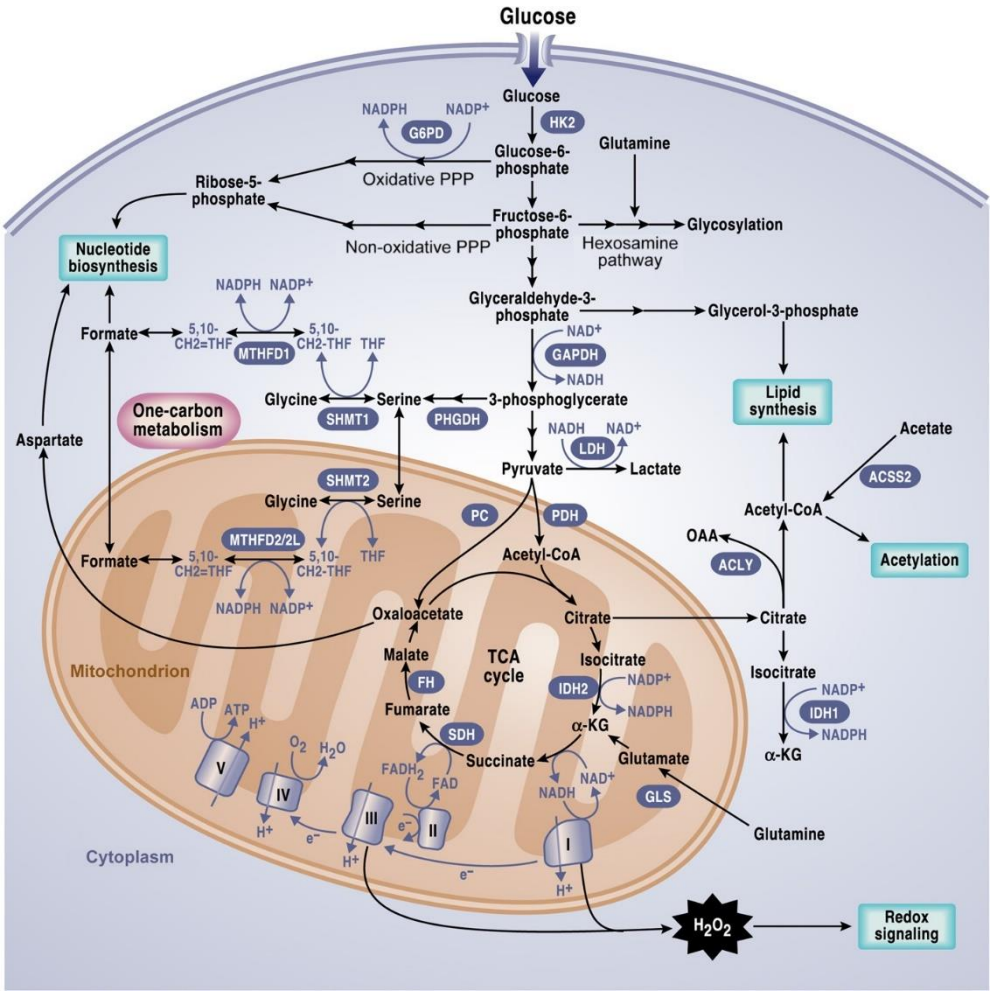

Figure 2: *The main metabolic pathways involved in cancer metabolic reprogramming (57).*

The classical example of metabolic reprogramming is the Warburg effect, or "aerobic glycolysis", defined as a regulated metabolic state in which cancer cells upregulate glucose uptake and generate lactate even in the presence of adequate oxygen levels to increase the nutrient import for anabolic processes (59). Initially, the increased glycolytic flux was interpreted as a consequence of mitochondrial dysfunction (60). Subsequently, it has emerged that mitochondria still remain functional in the majority of cancer cells and that respiration, and mitochondrial function in general, plays an important role in cancer (61).

Currently, it is clear that aerobic glycolysis, although an inefficient way to produce energy, generates several metabolic intermediates used by cancer cells for *de novo* synthesis of nucleotides, nonessential amino acids, and lipids (62). For example, glucose-6-phosphate can be processed through the pentose phosphate pathway producing ribose-5-phosphate, a structural component of nucleotides, and NADPH (Nicotinamide adenine dinucleotide phosphate). Fructose-6-phosphate can leave glycolysis and become a substrate for the hexosamine biosynthetic pathway, which generate substrates for protein glycosylation, one of the major post-translational modifications. Dihydroxyacetone phosphate can be converted into glycerol-3-phosphate used for biosynthesis of triacylglycerols and phospholipids, major components of cellular membranes. Finally, another glycolytic intermediate, 3-phosphoglycerate, serves as carbon source for the biosynthesis of nonessential amino acids serine and glycine, which participate into folate metabolism as one-carbon unit donor leading to the synthesis of purines and NADPH generation. NADPH resulting from glycolysis branched pathways is an important cofactor and provides reducing power for anabolic reactions and antioxidant systems by maintaining reduced glutathione and thioredoxin (63). Therefore, simultaneous increase of glycolysis and decrease of mitochondrial respiration allows cancer cells to balance their energetic needs with their ability to proliferate rapidly, supporting biomass accumulation and redox maintenance. Like glycolytic intermediates, TCA (tricarboxylic acid) cycle intermediates are also used as precursors for biosynthetic pathways and NADPH production (64). Indeed, TCA cycle not only produces reducing equivalents in terms of NADH and FADH2, which are used for ATP generation through oxidative phosphorylation (OXPHOS), but also provides building blocks for the synthesis of lipids and nucleotides, highlighting the important role of mitochondria in cancer cells proliferation and survival (65). In particular, the first intermediate of TCA cycle, citrate, can be exported into the cytosol and converted in acetyl-CoA and oxaloacetate by ATP-citrate lyase (ACLY). The cytosolic pool of acetyl-CoA promotes the fatty acid and cholesterol biosynthesis, while oxaloacetate sustains the production of aspartate required for nucleotide synthesis (66). To sustain the TCA cycle, different metabolic pathways replenish the cycle of intermediates in a process termed “anaplerosis”. Glutamine, serine and fatty acids are the main anaplerotic substrates. Glutamine can be captured in mitochondria and transformed into glutamate by the glutaminase enzyme, the resulting glutamate is converted into α-ketoglutarate to fuel the TCA cycle or alternatively is exported to the cytosol to participate in the biosynthesis of glutathione and nonessential amino acids, simultaneously producing NADPH. Moreover, glutamine-derived α-ketoglutarate can be reductively converted into citrate used to expand the cytosolic pool of acetyl-CoA and support de novo lipogenesis (67). Notably, glutamine-derived malate can be used to generate NADPH by the malic enzyme (68). Unsurprisingly, many cancer cells have shown a significant dependence on glutamine metabolism to proliferate and survive (67). Therefore, cancer cells can take advantage of using oxidizable substrates alternative to

glucose to fuel the TCA cycle and the OXPHOS, highlighting the important role played by mitochondria in metabolic adaptations that support tumor growth (69).

During tumor progression, cancer cells must acquire the ability to tolerate several stressful conditions, including oxygen and nutrients deprivation, to continue proliferating. For this purpose, cancer cells can use opportunistic mode of nutrient acquisition (56). Macropinocytosis allows cells to capture extracellular proteins, which undergo proteolytic degradation to liberate free amino acids, including glutamine, that can fuel the TCA cycle (70). Alternatively, cancer cells can recover nutrients from the digestion of entire living cells via entosis, or apoptotic bodies via phagocytosis (71);(72). Moreover, the nutrient scarcity favors metabolic cooperation among cancer cells and between cancer cells and nearby cells (i.e, stromal and immune cells) (73). For example, in human lung cancer the lactate generated by hypoxic cancer cells as end-product of glycolysis can be used by adjacent more oxygenated cells to fuel the TCA cycle and the OXPHOS (74). Another example of metabolic symbiosis is the so-called "reverse Warburg", in which ROS produced by cancer cells induce a pseudo-hypoxic environment in the tumor-associated fibroblasts, promoting glycolysis and lactic fermentation, which is used by cancer cells to increase mitochondrial respiration rate (75). At later stages of tumor development, cancer cells penetrate into the surrounding tissues, starting the invasion-metastasis cascade that continues with the intravasation of cells into blood and lymphatic vessels, the extravasation and colonization of distant tissues (76). Metabolic factors severely affect the ability of cancer cells to face the stressful conditions associated with the metastatic process. Increased mitochondrial biogenesis and functional OXPHOS activity seem to be an important requirement for metastatic dissemination in different tumor (77);(78);(79). Indeed, elevated levels of PGC-1α, the master regulator of mitochondrial biogenesis, and mitochondrial oxygen consumption rate have been found in circulating cancer cells compared to cancer cells from the primary tumor (77). Moreover, silencing of PGC-1α reduces the invasive and migratory properties of primary cancer cells (77). The increased mitochondrial functions observed in metastasizing cells and the oxidizing environment of the bloodstream generate a redox imbalance, and consequently the cancer cells rely on generation of NADPH and activation of antioxidant system to counteract the oxidative stress (80). The effects of ROS on cell fate depend on the balance between ROS generation and their scavenging in a stage- and tumor-specific contexts. At moderate levels, ROS can promote tumorigenesis by increasing cell proliferation, survival and mutation rate, and sustain tumor progression by promoting cancer cell motility (81). At high concentrations, ROS cause detrimental oxidative stress that damages macromolecules and lead to cell death (82). However, not all cancer cells are susceptible to oxidative stress in the same way. Reducing oxidative stress, by antioxidants or upregulating antioxidant enzymes, promotes metastasis in mouse models of melanoma, breast and lung cancer, while suppressing metastatic dissemination in other cancer models (77);(79);(83);(84). Overall, the metabolic phenotypes that stimulate and sustain

malignant transformation are generally distinct from those driving metastasis. Consequently, characterizing the evolution of metabolic properties acquired or lost by cancer cells during tumor development may allow to identify the stage-specific metabolic vulnerabilities offering new therapeutic opportunities.

1.3.2 Metabolic phenotype of drug-resistant ovarian cancer cells

Characteristics of cancer cells change under the selective pressure of therapeutic drugs through different mechanisms (85). In addition to the well-described genetic and epigenetics alterations, metabolic reprogramming has been proposed as an important mechanism that can induce the selection of pre-existing, intrinsically resistant subclones, or the evolution of drug-tolerant cells (86). Recently, it has been described that highly aggressive and drug resistant cancer cells often show an "hybrid glycolysis/OXPHOS phenotype", which allows them to switch between glycolysis and OXPHOS to produce energy and increase tumor growth (87). This phenotype is specifically associated with metastasis and drug resistance, since it enables cancer cells to reprogram their metabolic activities to maintain a metabolic fitness across progressing stages and stressful conditions (87).Cellular bioenergetic profile of patient-derived OC cell lines revealed that chemoresistant OC cells exhibit a highly active metabolic state, with enhanced OXPHOS and glycolytic rate, compared to their sensitive counterparts. Indeed, resistant cells are able to enhance the glycolytic flux in response to ATP synthase inhibitors, and increase the OXPHOS when glycolysis is inhibited (88). Moreover, OC stem cells, suggested to be responsible for drug resistance, also exhibit a hybrid phenotype with a preference for oxidative metabolism (61). Several studies have shown the association between cisplatin resistance and increased OXPHOS activity in OC cell lines (89); (90). In this context, the molecular chaperone TRAP1 (Tumour necrosis factor Receptor-Associated Protein 1) has been identified as a key regulator of metabolic remodelling in OC progression (91). TRAP1 is a heat shock protein (HSP) 90 family member with a prevalent, but not exclusive, mitochondrial localization that regulates the energetic metabolism of cancer cells (92). In particular, TRAP1 reduces OXPHOS by inhibiting complex II and IV of the respiratory chain (Fig. 3) (92);(93). Consequently, TRAP1 is mainly upregulated in cancer cells with a predominant glycolytic phenotype and downregulated in tumor cells relying on oxidative metabolism (94). Low TRAP1 expression levels correlate with more advanced disease in OC patients and drug-resistant OC cells showing increased oxidative metabolism (89). Moreover, TRAP1 knock down increases mitochondrial respiration and drives cisplatin resistance in OC cells (89). In particular, the metabolic shift towards OXPHOS, induced by TRAP1 downregulation, triggers the production of proinflammatory cytokines, such as IL6 and IL8, that in turn increases the expression of multi-drug resistance family members involved in platinum resistance of several cancer types (89). These observations support the tumor suppressive role played by TRAP1 in the aggressive stages of OC.

Figure 3: *TRAP1-mediated regulation of mitochondrial respiration. TRAP1 physically interacts with complex II of the mitochondrial respiratory chain, downregulating its activity. Moreover, TRAP1 also stabilizes the inactive form of c-Src which is known to stimulate complex IV activity.*

In support of the OXPHOS role in the onset of a chemoresistant phenotype, inhibition of respiratory complexes by metformin or oligomycin is able to reverse cisplatin resistance in OC cells and in patient-derived xenografts (89);(95). Notably, a significant upregulation of mitochondrial biogenesis and respiration following cisplatin exposure has been reported in different cancer cell lines (96);(97). This evidence strongly suggests that the high active metabolic state displayed by resistant cells may be induced by cisplatin treatment.In addition to a more oxidative metabolism, highly invasive OC cells have shown a deregulation of lipid metabolism (98). Rewiring of lipid metabolism significantly contribute to cancer progression in several human cancers, by serving as energy source and providing structural component of cellular membranes (99). Cholesterol plays an essential role in the regulation of physical and functional proprieties of cellular membranes by regulating membrane fluidity and signaling cascade initiation (100). Several studies have reported that cholesterol homeostasis is frequently deregulated in different tumor types, including OC (101). In particular, high cholesterol levels, due to increased cholesterol synthesis or uptake, promote tumor progression and drug resistance in OC (101). Indeed, increased expression of the rate-limiting enzyme of cholesterol, 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), and the downstream enzymes, Farnesyl-Diphosphate Farnesyltransferase 1 (FDFT1)

and Squalene Epoxidase (SQLE), have been correlated with poor overall survival and chemoresistance in OC patients (101). Moreover, high expression levels of low-density lipoprotein (LDL) receptor (LDLR), responsible for exogenous cholesterol uptake, have been found upregulated in drug-resistant OC cell lines (102). Several mechanisms have been described by which high intracellular cholesterol levels can contribute to drug resistance in cancer cells. For example, cholesterol reduces the sensitivity of OC cells to cisplatin and paclitaxel by increasing the expression of drug efflux pump proteins ABCG2 and MDR1 (103). Moreover, increased cholesterol content of plasma membrane decreases membrane permeability to cisplatin (104). For these reasons, the use of cholesterol-lowering drugs, such as statins, has been suggested as anticancer therapy (105). Simvastatin and lovastatin have been shown to reduce cancer cell proliferation, invasion and migration and promote chemotherapeutic sensitivity in *in vitro* and *in vivo* models of OC (106); (107);(108). However, anticancer and chemosensitizing proprieties of statins may be unrelated to the regulation of cholesterol homeostasis, considering their lipid-independent pleiotropic effects, including alteration of protein prenylation, inhibition of Ras family GTPase signaling, suppression of the PI3K/Akt/mTOR pathway (109). Furthermore, different epidemiological control studies have suggested that statin usage is not associated with an improved overall survival in OC patients (110). Therefore, the efficacy of lipid-lowering drugs for anticancer therapy is still controversial and needs investigations.

The remodelling of lipid metabolism in cancer cells is particularly influenced by the presence of adipocytes. OC cells predominantly disseminate through peritoneal fluid to omentum within the abdominal cavity (8). The omentum is mainly composed of adipocytes that generate a supportive microenvironment for metastatic progression and drug resistance (111). Indeed, omental adipocytes promote migration and invasion of OC cells by releasing adipokines, including IL-6, IL-8 and adiponectin, that attract the cells to omentum, and sustain cancer cell proliferation by inducing the PI3K/AKT pathway (112);(113). Once OC cells reach the omental fat, a bidirectional crosstalk is established between cancer cells and cancer-associated adipocytes, which leads to metabolic changes of both cell types that sustain tumor progression (111). In particular, cancer cells induce the activation of lipolysis in adipocytes and use the fatty acids released in the environment as energy source by increasing mitochondrial β-oxidation and thus OXPHOS for ATP production (112). Accordingly, metastatic OC cells increase fatty acid uptake, by upregulating the fatty acid receptor CD36, and utilization by increasing the expression of the lipid chaperone FABP4 and CPT1, the rate-limiting enzyme for fatty acid oxidation (114);(115);(116). The metabolic synergism shown by OC cells and adipocyte through reprogramming of lipid metabolism not only enhance metastatic progression but also contribute to development of drug resistance (117). Indeed, the inhibition of FABP4 reduces tumor burden in mouse models and increase the sensitivity of OC cells to platinum compounds (115).

7. LIST OF PUBLICATIONS

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