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In vitro Cytotoxic Activity of Flavonoids on Human Ovarian Cancer Cell Lines

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Abstract

Ovarian carcinoma remains one of the most fatal female malignancies representing the fifth leading cause of cancer deaths in women. The progress in prevention, early diagnosis and treatment of this devastating disorder has been limited to date and therefore, the development of new treatment options is highly needed. In this review article, data about the *in vitro* cytotoxic action of naturally occurring flavonoids in various human ovarian cancer cell lines are compiled and analyzed, showing the growth inhibitory effects both in chemosensitive as well as chemoresistant cells. Anticancer action of these compounds is mediated through different cellular mechanisms including induction of apoptosis and cell cycle arrest, inhibition of cellular migration and invasion, suppression of expression of vascular endothelial growth factor, and triggering the non-apoptotic cell death. Also, estrogen receptors mediated mechanisms can be involved in the tumoricidal responses to flavonoids. As the resistance to conventional chemotherapy drugs is the most significant cause of treatment failure, the ability of several flavonoids to sensitize ovarian cancer cells to these drugs may have an important clinical significance and therapeutic applications in the management of ovarian tumors.

Keywords: Ovarian cancer; Natural flavonoids; Anticancer mechanisms; Estrogen receptors; Chemosensitization

Introduction

Ovarian cancer affects about 1-2% of females in their lifetime developing in one of 70 women. One woman in 100 will lose her life due to the disease related complications [1,2]. Altogether more than 204,000 new cases are diagnosed each year and an estimated 130,000 deaths worldwide [3-6]. The incidence of ovarian cancer has risen over the past century and continues to grow being highest in Europe and Northern America and somewhat lower in Japan and less developed countries [7-9].

Ovarian carcinoma accounts for about 4% of all female cancer cases being the sixth most commonly occurring cancer and the fifth leading cause of cancer deaths in women [3,6,10,11]. Among all gynecological malignancies ovarian cancer remains the most dreaded diagnosis with the highest mortality rate worldwide [1,4,12-14]. Progress in the early diagnosis and treatment has been limited, leaving survival and death rate of ovarian cancer unchanged over decades [14-16]. In more than two-thirds of patients this highly metastatic disease is diagnosed at advanced stages when cancer is spread beyond the ovaries and disseminated in the abdominal and peritoneal cavity [4,5,17-20]. The five year survival rate of patients with advanced disease (stages III-IV) is only around 20%, whereas the survival is close to 90% when the cancer is detected at stage I [3,17,21,22].

The primary cause for this high mortality rate is the failure to detect ovarian cancer at an initial stage. Difficulties in early detection are associated with the absence of effective population screening methods and specific signs and symptoms characteristic for initial stages of disease [23-27]. The risk of developing ovarian cancer increases with age; other risk factors include inflammations, family history, nulliparity, early menarche and late menopause [4,10,19]. Approximately 80-90% of ovarian cancer cases are epithelial, whereas the heterogenic nature of tumor also confers a poor prognosis and high lethality [4,5,28].

The standard management of ovarian cancer includes extensive surgical cytoreduction followed by combination chemotherapy using a taxane (paclitaxel) and platinum (cisplatin or carboplatin) containing regimen [3,5,9,28]. Although 70-80% of patients initially respond to the first-line chemotherapy, the majority (over 80%) will recur with chemoresistant phenotype within two years and ultimately die of metastatic disease [1,3,9,18,23,25,29-33]. Thus, the emergence of resistance to available chemotherapeutic drugs remains a major impediment to treatment success of recurrent ovarian cancer [3-5,28]. Moreover, a variety of cytotoxic agents are known to cause severe toxicity also to normal cells [10,17,34]. Therefore, the development of novel therapies to overcome chemoresistance and finding natural drugs with little toxicity to healthy tissues are urgent needs for successful treatment of ovarian cancer and improved overall survival of patients [3,5,10,12,17,28,34].

Chemoresistance is mostly caused by the defects in apoptotic machinery and targeting of apoptotic blockers may represent a promising approach for management of chemoresistant tumors [21,35,36]. Another way to reverse this resistance is to circumvent apoptosis and execute cell death through alternative non-apoptotic pathways [29,37]. The high rate of recurrence may also be caused by the survival of a small subset of slow-dividing and chemoresistant cells, the ovarian cancer stem cells which are able to regenerate the bulk of the tumor following

chemotherapy [17,25,38]. Therefore, development of new drugs to specifically kill these cells is vital to improve rates of tumor remission and increase survival of ovarian cancer patients. Also angiogenetic, migratory and invasive factors are exciting and perspective targets for pharmacological interventions of ovarian malignancies [39,40].

Prevention of ovarian cancer remains a challenging task because no specific carcinogens are known and no effective biomarkers for screening and early detection are available [16,19]. However, as only 5-10% of all ovarian cancers are hereditary the studies of environmental factors including the role of diet and specific dietary constituents appear attractive for both prevention as well as treatment of this disease [2,16,41]. Also, the geographic variation in incidence rates argues for an important role of modifiable factors like diet in ovarian tumorigenesis. Indeed, the incidence of ovarian cancer is highest in women living in Europe and North America and lowest among women in Japan being possibly related to the higher intake of soy isoflavones in Asian populations [14,28,42,43]. However, diets high in saturated fats and low in fruits and vegetables have been consistently associated with increased ovarian cancer risk [19,44,45]. Certain compounds in plant-based diets may be important in reducing the disease risk as an impressive 40% decrease in ovarian cancer incidence was found for individuals with the highest quintile of kaempferol intake suggesting this compound as a significant chemopreventive agent [15,16,43,44,46]. Also, drinking green tea has been associated with both decreased occurrence as well as better prognosis of ovarian cancer [8,23,27,47].

Effects of Flavonoids on Ovarian Cancer Cells

Natural products have played an important role in the discovery of anticancer agents: about 60% of cytotoxic drugs currently employed in cancer chemotherapy are derived from plant sources and interest in finding novel bioactive phytochemicals is still active [48-51].

Flavonoids as plant pigments comprise a class of natural phytochemicals displaying many biological activities [52,53]. More than 5000 individual flavonoids have been discovered, widely distributed in fruits, vegetables and medicinal herbs [18,53,54]. These polyphenolic compounds possess a common phenylbenzopyrone skeleton (C6-C3-C6) and are divided into various classes according to similarities in their structure; the main groups are flavanols, flavanones, flavones, flavonols, isoflavones, and anthocyanidins [18,43,46,53-55]. Flavonoids express a wide variety of biological effects that may play a role in both cancer prevention and also cancer therapy. These secondary metabolites reveal potent antiproliferative, antioxidant, antiangiogenic, and anti-inflammatory properties, induce apoptosis, and perturb cell cycle progression [10,19,53-55]. The exact effect of flavonoids in certain systems depends on several factors including their concentrations and cell lines; however, despite the promising preclinical results the possible therapeutic application of these plant polyphenols is hampered by their low bioavailability [15,56,57].

To study the possible anticancer effects of flavonoids on ovarian tumorigenesis many different cell lines have been used. The overview of these lines with their principal characteristics is presented in Table 1. Data about the cytotoxic activity of flavonoids in malignant ovarian cells are compiled in Table 2.

Flavanols and Catechins

The polyphenolic catechin epigallocatechin gallate (EGCG) is the major active constituent of green tea accounting for 50-80% of its total catechins content [7,20,27]. EGCG has been shown to inhibit the growth of various ovarian cancer cell lines including SKOV3, CaOV3, OVCAR3, PA-1, HEY, OVCA 433, A2780 and its chemoresistant sublines [7,20,39,47,58,59]. This inhibition is dose- and time-dependent and is expressed both in chemosensitive as well as chemoresistant cell lines (Table 2). The mean growth inhibitory constant by incubating the cells for 72 h can be calculated as 5.85 ± 0.98 μM (n = 8). Although the exact mechanisms of this anticancer action are not fully understood, EGCG can suppress the ovarian cancer cell growth through induction of apoptosis, arresting the cell cycle in G1 or G1/S phase, and regulating the gene expression [7,8,39,47,58-60] (Figure 1).

Besides EGCG other green tea catechins are also found to be effective against ovarian cancer cells, whereas epicatechin gallate (ECG) is even more potent growth inhibitor than EGCG in two epithelial ovarian cancer cell lines HH450 and HH639 [61]. Furthermore, oral administration of green tea extract to nude mice bearing HEY ovarian carcinoma xenografts can induce a significant reduction in tumor growth being associated with inhibition of neovascularization [39]. Thus, green tea polyphenols may be useful in suppressing the progression of ovarian carcinoma and are certainly worthy further studies for possible chemotherapeutic applications.

Flavones

Different flavones exert rather diverse effects on human ovarian cancer cells (Table 2). The widely distributed flavonoid apigenin has only a weak antiproliferative activity on OVCAR3 and A2780 cells; however, this compound is able to significantly inhibit invasion and migration of tumor cells suppressing the metastatic progression of ovarian carcinoma [13,31,45,62]. High micromolar growth inhibitory effects have been described also for baicalein and baicalin, wogonin, hispidulin, and jaceosidin in several ovarian cancer lines [4,19,21,51]. Due to the considerably higher inhibitory effect of wogonin on paclitaxel-resistant subline PTX10 compared to its parent chemosensitive line A2780 this flavone may be an attractive therapeutic candidate for treatment of chemoresistant tumors [4].

Several flavones such as apigenin, baicalein and baicalin are able to inhibit the expression of Vascular Endothelial Growth Factor (VEGF) and it is obvious that targeting this molecule may be a promising strategy for further treatment of ovarian cancers [19,62]. In the case of apigenin, it has been demonstrated that this compound can display anticancer activity also *in vivo* experiments by inhibiting the metastasis of A2780 and OVCAR3 cells injected into the ovaries of nude mice [13,31]. These results

Table 1: Characterization of human ovarian cancer cell lines used for cytotoxicity studies with flavonoids.

Parental line	Subline	Characterization
2008		Sensitive to cisplatin
	2008/C13	Resistant to cisplatin; wt p53
A2780		Sensitive to cisplatin and paclitaxel; wt p53; established from tumor tissue of an untreated patient
	$A2780^{cisR}$; $A2780^{cp}$; C30; C200; CP70	Resistant to cisplatin
	$A2780^{P}$; A2780/taxol; $A2780^{TR}$; PTX10	Resistant to paclitaxel
	$A2780^{ZD0473R}$	Resistant to ZD0473
BG-1		Established from a stage III poorly differentiated adenocarcinoma
	AS4	BRCA1 blocked
	NEO	BRCA1 unblocked
CaOV3		Resistant to cisplatin; established from adenocarcinoma of a 54-years-old Caucasian female
COC1		
EFO27		Resistant to paclitaxel
ES2		Resistant to cisplatin
HEY		
HH450		Established from moderately differentiated metastatic cells recovered from the abdominal fluid of a 52-year-old Asian female
HH639		Established from a poorly differentiated clear cell, Grade 3 carcinoma in the omentum and left ovary of a 56-year-old Caucasian female
HO-8910		Low metastatic potential cells
MDAH-2774		
MPSC1		
	$MPSC1^{TR}$	Resistant to paclitaxel
OVCA 429		
OVCA 433		
OVCAR3		Resistant to cisplatin and paclitaxel; established from the malignant peritoneal ascites of a patient with poorly differentiated progressive papillary ovarian adenocarcinoma; mutant p53
OVCAR5		
OVCAR10		
PA-1		wt p53
RMUG-L		
SKOV3		Resistant to several cytotoxic drugs, incl diphtheria toxin, cisplatin, adriamycin and paclitaxel; established from the peritoneal ascitic fluid of a patient with ovarian serous adenocarcinoma of Grade 2/3; p53 null
	SKOV3-ip1	Sensitive to paclitaxel; more invasive and metastatic than parental cell line
	$SKOV3^{TR}$; $SKOV3^{TR}$-ip2	Resistant to paclitaxel
TOV-21G		
UL-3C		Established form stage IIIc ovarian cancer
UL-5		Established form stage IIIc ovarian cancer
UL-6		Established form stage IIIc ovarian cancer
UL-7		Established form stage IIIc ovarian cancer
UL-8		Established form stage IIIc ovarian cancer

Table 2: Cytotoxic effects of common natural flavonoids on human ovarian cancer cell lines.

Flavonoid	Cell line	Cytotoxic activity	Assay method/ time	
Flavanols				
Epicatechin gallate (ECG)	HH450	IC_{50} 28.95 μM	Cell counting	[61]
	HH639	IC_{50} 29.59 μM	Cell counting	[61]
Epigallocatechin gallate (EGCG)	A2780	IC_{50} 2.5 μM	Alamar Blue Assay/ 4 days	[20]
		IC_{50} 4.46 ± 0.34 μM	MTT cell viability assay/ 72 h	[27]
		IC_{50} 6.87 ± 2.72 μM	MTT cell viability assay/ 72 h	[5]
	$A2780^{cisR}$	IC_{50} 5.90 ± 0.81 μM	MTT cell viability assay/ 72 h	[27]
		IC_{50} 6.67 ± 3.61 μM	MTT cell viability assay/ 72 h	[5]
	$A2780^{ZD0473R}$	IC_{50} 9.63 ± 4.73 μM	MTT cell viability assay/ 72 h	[5]
		IC_{50} 11.80 ± 0.72 μM	MTT cell viability assay/ 72 h	[27]
	A2780/C200	IC_{50} 15 μM	Alamar Blue Assay/ 4 days	[20]
	A2780/C30	IC_{50} 7.5 μM	Alamar Blue Assay/ 4 days	[20]
	A2780/CP70	IC_{50} 2.5 μM	Alamar Blue Assay/ 4 days	[20]
	CaOV3	IC_{50} 15 μM	Alamar Blue Assay/ 4 days	[20]
	HEY	IC_{50} 20 μM; induction of apoptosis	Trypan blue dye exclusion/ 24 h	[39]
	HH450	IC_{50} 62.25 μM	Cell counting	[61]
	HH639	IC_{50} 42.21 μM	Cell counting	[61]
	OVCA433	IC_{50} 20 μM; induction of apoptosis	Trypan blue dye exclusion/ 24 h	[39]
	OVCAR3	IC_{50} 4.5 μM	Alamar Blue Assay/ 4 days	[20]
		Inhibition at doses of 10 to 100 μM; induction of G1 phase cell cycle arrest and apoptosis	Cell counting/ various times	[58]
		Inhibition at doses of 10 to 100 μM; induction of G1 phase cell cycle arrest and apoptosis	Cell counting/ various times	[59]
	OVCAR10	IC_{50} 7.5 μM	Alamar Blue Assay/ 4 days	[20]
	PA-1	Inhibition at doses of 10 to 100 μM; induction of G1/S phase cell cycle arrest and apoptosis	Cell counting/ various times	[58]
		Inhibition at doses of 10 to 100 μM; induction of G1/S phase cell cycle arrest and apoptosis	Cell counting/ various times	[59]
	SKOV3	IC_{50} 11.08 ± 1.21 μM	MTT cell viability assay/ 72 h	[5]
		IC_{50} 15 μM	Alamar Blue Assay/ 4 days	[20]
		IC_{50} 21.0 ± 2.0 μM; induction of apoptosis	MTT cell viability assay/ 72 h	[60]
		IC_{50} 31 ± 1.5 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[60]
		IC_{50} 98.8 ± 3.6 μM; induction of apoptosis	MTT cell viability assay/ 24 h	[60]
		50.67 ± 2.40% inhibition at 87.27 μM; induction of apoptosis	MTT cell viability assay/ 24 h	[47]
		Significant inhibition at doses > 65.5 μM; induction of G1 phase cell cycle arrest, apoptosis	MTS cell viability assay/ 2 days	[7]
		Inhibition at doses of 10 to 100 μM; induction of G1 phase cell cycle arrest and apoptosis	Cell counting/ various times	[58]
		Inhibition at doses of 10 to 100 μM; induction of G1 phase cell cycle arrest and apoptosis	Cell counting/ various times	[59]
	SKOV3-ip1	No effect up to 30 μM	MTT cell viability assay/ 24, 48, 72 h	[23]
	$SKOV3^{TR}$-ip2	No effect up to 20 μM	MTT cell viability assay/ 24, 48, 72 h	[23]
Flavones				
Apigenin	A2780	Some inhibition at 20 and 40 μM; induction of G2/M phase cell cycle arrest and apoptosis	Cell counting/ 24, 48 h	[91]
		Small effect at 20 and 40, some inhibition at 60 μM; inhibition of cell migration, invasion	MTT cell viability assay/ 16, 24 h	[31]
	A2780/CP70	No effect at 5-15 μM; inhibition of VEFG	MTT cell viability assay/ 15 h	[62]
	OVCAR3	Inhibition to 21% by 160 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[45]
		Some effect at doses > 45 μM	MTS cell viability assay/ 24 h	[30]
		No effect at 10 μM; inhibition of cell migration and invasion		[13]

		Some inhibition at 20 and 40 μM	Cell counting/ 24, 48 h	[91]
		Inhibition of migration and invasion		[31]
		No effect at 5-15 μM; inhibition of VEGF	MTT cell viability assay/ 15 h	[62]
Baicalein	A2780/CP70	IC_{50} 24.3 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[19]
	OVCAR3	IC_{50} 39.4 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[19]
Baicalin	A2780/CP70	IC_{50} 55.2 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[19]
	OVCAR3	IC_{50} 44.6 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[19]
Ginkgetin	OVCAR3	IC_{50} 3.18 μM	Trypan blue dye exclusion/ 48 h	[63]
		IC_{50} 5.30 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[64]
Hispidulin	SKOV3	Minimal effect at < 20 μM	MTT cell viability assay/ 48 h	[21]
Jaceosidin	CaOV3	Significant inhibition at >10 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[51]
	SKOV3	Significant inhibition at > 10 μM	MTT cell viability assay/ 48 h	[51]
Luteolin	OVCAR3	Inhibition to 21% by 160 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[45]
		Cytotoxic effect at > 30 μM	MTT cell viability assay/ 24 h	[30]
Protoapigenone	MDAH-2774	IC_{50} 0.69 ± 0.92 μM; induction of S and G2/M phase cell cycle arrest and apoptosis	XTT cell viability assay/ 48 h	[55]
	SKOV3	IC_{50} 0.78 ± 0.28 μM; induction of S and G2/M phase cell cycle arrest and apoptosis	XTT cell viability assay/ 48 h	[55]
Scutellarein	OVCAR3	< 10% inhibition at 160 μM	MTT cell viability assay/ 24 h	[30]
Scutellarin	OVCAR3	Some cytotoxicity at > 45 μM	MTT cell viability assay/ 24 h	[30]
Wogonin	A2780	IC_{50} 24.63 ± 1.41 μM; induction of G1 phase cell cycle arrest and apoptosis	MTS cell viability assay/ 96 h	[4]
	A2780/PTX10	IC_{50} 6.44 ± 3.06 μM; induction of G2/M phase cell cycle arrest and apoptosis	MTS cell viability assay/ 96 h	[4]
	OVCAR3	Some cytotoxicity at > 45 μM	MTT cell viability assay/ 24 h	[30]
Flavonols				
Kaempferol	A2780	No effect at 20 μM; inhibition at > 40 μM; induction of apoptosis	SYBR green assay/ 24 h	[16]
	A2780/CP70	Inhibition to 94 and 79% by 40 and 80 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[44]
		No effect at 25 μM; induction of apoptosis	MTS cell viability assay/ 24 h	[15]
		No effect at 20 μM; inhibition at > 40 μM; induction of apoptosis	SYBR green assay/ 24 h	[16]
	OVCA 429	No effect up to 100 μM	MTT cell viability assay/ 72 h	[26]
	OVCAR3	Constricted inhibition (> 60%) at 160 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[45]
		Inhibition to 91 and 74% by 20 and 80 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[44]
		Some inhibition at 25 μM	MTS cell viability assay/ 24 h	[15]
		No effect at 20 μM; inhibition at > 40 μM; induction of apoptosis	SYBR green assay/ 24 h	[16]
	RMUG-L	No effect up to 100 μM	MTT cell viability assay/ 72 h	[26]
	SKOV3	IC_{50} 28.65 ± 1.05 μM	SRB cell viability assay/ 48 h	[92]
Quercetin	A2780	IC_{50} 22.69 ± 3.86 μM	MTT cell viability assay/ 72 h	[34]
		About 56.3% inhibition at 99.34 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[10]
		Significant inhibition; induction of G0/G1 and G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 48 h	[1]
	$A2780^{cisR}$	IC_{50} 25.95 ± 5.34 μM	MTT cell viability assay/ 72 h	[34]
	$A2780^{cp}$	Significant inhibition; induction of G0/G1 and G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 48 h	[1]
	$A2780^{P}$	IC_{50} 70 μM	SRB cell viability assay/ 4 days	[9]
	CaOV3	Inhibition at doses 10-20 μM	Cell counting/ 3 days	[65]
	EFO27	IC_{50} 59 μM	SRB cell viability assay/ 4 days	[9]
	OVCA 429	No effect up to 10 μM	MTT cell viability assay/ 72 h	[26]
	OVCA 433	Induction of G0/G1 phase cell cycle arrest	Cell counting/ 72 h	[66]

	OVCAR3	IC_{50} 42 μM	SRB cell viability assay/ 4 days	[9]
		IC_{50} 217.2 ± 4.89 μM; induction of apoptosis	Cell counting/ 24 h	[50]
		Slight inhibition at < 20 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[45]
		Slight inhibition at 160 μM	MTT cell viability assay/ 24 h	[30]
	OVCAR5	IC_{50} 66 ± 3.0 μM	Cell counting/ 3 days	[87]
	RMUG-L	No effect up to 100 μM	MTT cell viability assay/ 72 h	[26]
	SKOV3	IC_{50} 20.84 ± 0.66 μM	SRB cell viability assay/ 48 h	[92]
		IC_{50} 90 μM	SRB cell viability assay/ 4 days	[9]
		IC_{50} 222.1 ± 5.64 μM; induction of apoptosis	Cell counting/ 24 h	[50]
		Inhibition at doses of 10-20 μM	Cell counting/ 3 days	[65]
	TOV-21G	IC_{50} 237.6 ± 6.07 μM; induction of apoptosis	Cell counting/ 24 h	[50]
Rutin	OVCA 433	No effect up to 10 μM	Cell counting/ 72 h	[66]
	OVCAR3	No effect up to 160 μM	MTS cell viability assay/ 24 h	[45]
Isoflavones				
Genistein	A2780	Induction of apoptosis and autophagocytosis	SRB cell viability assay/ 24-96 h	[29]
	A2780/CP70	IC_{50} 38.85 μM	CellTiter assay/ 24 h	[89]
	BG-1/AS4	IC_{50} 165.3 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[82]
	BG-1/NEO	IC_{50} 171 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[82]
	CaOV3	Induction of apoptosis and autophagocytosis	SRB cell viability assay/ 24-96 h	[29]
	COC1	IC_{50} 51.41 ± 3.6 μM; induction of G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 48 h	[32]
	ES2	Induction of apoptosis and autophagocytosis	SRB cell viability assay/ 24-96 h	[29]
		No effect up to 10 μM	Crystal violet assay/ 48 h	[68]
	HO-8910	30.7 and 68.0% inhibition at 50 and 100 μM; G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 72 h	[6]
	OVCAR3	Constricted inhibition (> 60%) at 160 μM; inhibition of VEGF	MTS cell viability assay/ 24 h	[45]
	SKOV3	IC_{50} 48.2 ± 4.6 μM; induction of G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 48 h	[32]
		Some inhibition at micromolar doses; induction of G2/M phase cell cycle arrest, apoptosis	MTT cell viability assay/ 24, 48 h	[52]
		About 72% inhibition at 200 μM; inhibition of cell migration	SRB cell viability assay/ 72 h	[12}
		Inhibition to 58% by 200 μM; inhibition of cell migration	SRB cell viability assay/ 48 h	[12]
		No effect up to 10 μM	Crystal violet assay/ 48 h	[68]
	UL-3C	Inhibition at micromolar doses	SRB cell viability assay/ 96 h	[14]
	UL-5	Inhibition at micromolar doses	SRB cell viability assay/ 96 h	[14]
	UL-6	IC_{50} 27 μM	SRB cell viability assay/ 96 h	[14]
	UL-7	Inhibition at micromolar doses	SRB cell viability assay/ 96 h	[14]
	UL-8	IC_{50} 148 μM	SRB cell viability assay/ 96 h	[14]
Genistin	SKOV3	28% inhibition at 50 μM; induction of G1 and G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 24, 48 h	[52]
Tectorigenin	A2780	IC_{50} 108.2 ± 12.9 μM	MTT cell viability assay/ 48 h	[3]
	$A2780^{TR}$	IC_{50} 78.8 ± 5.8 μM	MTT cell viability assay/ 48 h	[3]
	MPSC1	IC_{50} 123.4 ± 13.4 μM	MTT cell viability assay/ 48 h	[3]
	$MPSC1^{TR}$	IC_{50} 73.1 ± 12.2 μM	MTT cell viability assay/ 48 h	[3]
	SKOV3	IC_{50} > 200 μM	MTT cell viability assay/ 48 h	[3]
	$SKOV3^{TR}$	IC_{50} 89.6 ± 10.2 μM	MTT cell viability assay/ 48 h	[3]
Flavanones				
Hesperidin	OVCA 433	No effect up to 10 μM	Cell counting/ 72 h	[66]
Naringin	OVCAR3	No effect up to 160 μM	MTS cell viability assay/ 24 h	[45]
Tangeretin	2008/C13	IC_{50} 238.3 μM	MTT cell viability assay/ 72 h	[69]
	A2780/CP70	IC_{50} 239.7 μM	MTT cell viability assay/ 72 h	[69]
Isoxanthohumol	A2780	IC_{50} 18.0 μM	SRB cell viability assay/ 2 days	[71]
		IC_{50} 25.7 μM	SRB cell viability assay/ 4 days	[71]

Flavanonols				
Taxifolin	OVCAR3	Constricted inhibition (> 60%) at 160 μM	MTS cell viability assay/ 24 h	[45]
Flavanolignans				
Silibinin	A2780	Up to 55% inhibition at 200 μM	MTT cell viability assay/ 72 h	[70]
		About 58-65% viability at 50 μM; induction of apoptosis	MTT cell viability assay/ 48 h	[18]
		No effect at 10 μM	ATP cell viability assay/ 72 h	[57]
	A2780cp	No effect at 10 μM	ATP cell viability assay/ 72 h	[57]
	A2780/taxol	Up to 58% inhibition at 200 μM; G1 and G2/M phase cell cycle arrest and apoptosis	MTT cell viability assay/ 72 h	[70]
	SKOV3	About 58-65% viability at 50 μM; induction of apoptosis	MTT cell viability assay/ 48h	[18]
Chalcones				
Isoliquiritigerin	SKOV3	83.08% inhibition at 156.10 μM; induction of apoptosis	MTT cell viability assay/ 24 h	[72]
Xanthohumol	A2780	IC_{50} 0.52 μM	SRB cell viability assay/ 2 days	[71]
		IC_{50} 5.22 μM	SRB cell viability assay/ 4 days	[71]
		IC_{50} 8.7 μM	SRB cell viability assay/ 6 days	[71]

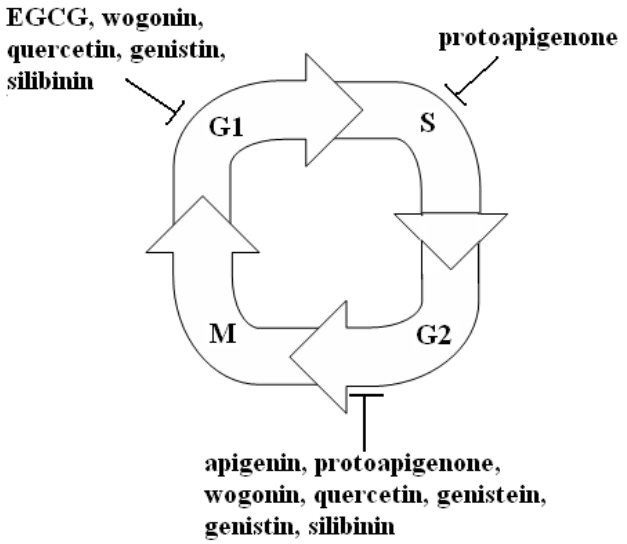

Figure 1: Perturbation of cell cycle progression by flavonoids in human ovarian cancer cells (data derived from Table 2).

are especially important bearing in mind the highly metastatic nature of this disease.

Considerably stronger cytotoxicity has been characterized for biflavone ginkgetin [63,64] and a novel natural flavone protoapigenone; this effect is mediated by induction of apoptosis and cell cycle arrest [55] (Figure 1). Moreover, the growth inhibitory action of protoapigenone but also baicalein and baicalin displays an apparent selectivity toward malignant cells as their effect on normal ovarian cells is significantly less [19,55]. In addition, treatment of nude mice implanted with MDAH-2774 human ovarian cancer cells with protoapigenone can lead to an important reduction in tumor sizes, concomitantly exerting no major side effects [55].

Flavonols

An abundant natural flavonol quercetin is the major contributor to dietary intake of flavonoids; however, its effects on ovarian cancer are rather rarely studied [10,54]. Although quercetin can inhibit the growth of several ovarian cancer cell lines (Table 2) these activities reveal mostly at concentrations higher than 10-20 μM being usually not attainable in physiological conditions [9,10,45,50,65]. This growth inhibition is typically accompanied by apoptotic changes and cell cycle arrest in G0/G1 and/or G2/M phases depending on the certain cell types [1,10,50,66,67] (Figure 1). Importantly, the inhibitory action of quercetin is similarly active in various cell lines including both chemosensitive as well as chemoresistant lines [1,9]. Moreover, this flavonol can exhibit significant anticancer activity also *in vivo* experiments by decreasing the tumor volume in nude mice injected with either cisplatin-sensitive or -resistant A2780 cells [1,10].

Bioavailability of hydrophobic flavonoids can be improved by their encapsulation into nanoparticles and indeed, PEGylated liposomal quercetin is slightly more cytotoxic than free quercetin in A2780 parent line and its cisplatin-resistant subline [1]. Also, the other widely distributed dietary flavonol kaempferol exerts only a constricted inhibition up to 30-40 μM doses in several ovarian cancer cell lines; however, nanoparticles incorporating this flavonoid reveal significantly greater inhibitory action compared to free kaempferol in A2780/CP70 and OVCAR3 cells [15,16,45]. Although kaempferol is low in direct cytotoxicity it can substantially inhibit the expression of VEGF suppressing the angiogenesis and repressing thus the tumor growth indirectly [15,44].

In contrast to flavonol aglycones the glycosidic derivative of quercetin, rutin is shown to be ineffective in different ovarian cancer cell lines [45,66].

Isoflavones

Similarly to common flavones and flavonols also isoflavones exert their antiproliferative effects at rather high concentrations, greater than 20 μM [14,22]. Genistein is the best known isoflavone being an important bioactive component of dietary soybean commonly consumed in Asian countries. However, its cytotoxic mechanisms in human ovarian cancer cells are still not thoroughly clear being possibly multifactorial [6,14,68].

Genistein has shown to inhibit the growth of both platinum sensitive as well as resistant cell lines in a dose- and time-dependent manner [12,28,29,45,52]. Moreover, the growth inhibitory effects of another isoflavone tectorigenin have found to be even more potent in taxane-resistant lines compared to their parental lines [3]. In addition to growth inhibition, genistein can decrease also the migration capacity of tumor cells offering thus a novel insight into its therapeutic action [12].

Like genistein, also its glycosidic derivative genistin is able to inhibit the proliferation of SKOV3 ovarian cancer cells being still considerably less potent [52]. Although both compounds induce apoptotic cell death and cell cycle arrest, genistein can block the cell cycle in G2/M phase in different ovarian cancer cell lines [6,32,52], whereas genistin arrests the cell cycle in both G2/M and G1 phases [52] (Figure 1). Genistein is able to initiate not only apoptosis but induces also autophagic cell death in ovarian cancer cells revealing an attractive mechanism to bypass chemoresistance caused by dysregulations in apoptotic pathways [29]. Therefore, genistein may become a useful anticancer drug for treatment of ovarian tumors.

Flavanones, flavanonols and other flavonoids

As presented in Table 2 three common flavanones hesperidin, naringin and tangeretin, and flavanonol taxifolin are either ineffective or express only very weak antiproliferative activity at high micromolar doses on malignant ovarian cells [45,66,69].

Also, flavanolignan silibinin from milk thistle is practically unable to produce growth inhibitory effect on ovarian cancer cells within physiologic concentration range [18,57,70].

On the other hand, several hop flavonoids express cytotoxic activity in ovarian cancer cells, whereas chalcone xanthohumol can inhibit the growth of A2780 cells even at high nanomolar doses. However, the respective flavanone isomer, isoxanthohumol is still less potent [71]. Some cytotoxic activity has shown for licorice chalcone isoliquiritigenin in SKOV3 cells by triggering oxidative stress and inducing apoptosis [72].

Ovarian Cancer is an Estrogen Dependent Tumor

Ovarian carcinoma is generally accepted as hormone responsive cancer and estrogens can probably play an important role in the development and progression of ovarian malignancies [2,3,42,73-77]. The actions of estrogens are typically mediated through two Estrogen Receptors (ER), ERα and ERβ, whereas approximately 63% of ovarian epithelial carcinomas are ER positive [2,3,78]. The ratio of these two receptor variants (ERα/ERβ) increases within tumorigenesis leading to either ERα overexpression or selective growth advantage of ERα positive cells [3,78]. At the same time the clinical implications of ERs in ovarian carcinogenesis including prognostic significance and potential antiestrogen treatment remain controversial and unclear [14,77,78].

Naturally occurring nonsteroidal plant compounds, phytoestrogens can reveal some estrogenic activity thus influencing the growth of hormone dependent tumors [3,79,80]. Such polyphenolic compounds may suppress ovarian cancer development via complex mechanisms through competitive binding to ERs or type II Estrogen Binding Sites (EBS); by decreasing endogenous bioavailable estrogen levels; by inhibiting ERα expression; and/or through reducing the activity of aromatase enzyme that converts androgens to estrogens [42,81].

For instance, the antiproliferative action of isoflavone genistein is considered to be mediated at least in part via ER dependent pathways [2,75,82]. It is thus possible that the variations in growth inhibitory effects of genistein in various ovarian cancer cell lines can reflect the differences in expression level of ER subtypes and possibly also the different inhibition modes (IC_{50} values for BG-1/AS4 165.3 μM, BG-1/NEO 171 μM, COC1 51.4 μM, and SKOV3 48.2 μM by incubating the cells for 48 h; Table 2). It is indeed well known that genistein has about 30 fold greater affinity for ERβ compared to ERα [82]. Besides classical ERs the type II estrogen binding sites are also described in ovarian cancer cells being able to bind flavonoids such as quercetin and regulate in this way the tumor growth [66,81,83].

On the other hand, green tea catechin EGCG has shown to be able to decrease the levels of circulating estrogens [7], while widely distributed dietary flavonol kaempferol can inhibit the expression of ERα in breast cancer cells [15,44] pointing altogether to the high complexity of anticancer action of flavonoids in living systems.

Sensitization of Cancer Cells to Chemotherapy Drugs

In addition to the anticancer effects expressed by flavonoids alone these compounds are also able to influence the action of conventional chemotherapeutic drugs.

The development of resistance to chemotherapy remains a major limitation in the treatment of ovarian cancer contributing to poor prognosis and high mortality. The main clinical problem involves the emergence of secondary or acquired resistance appearing after the response to front-line drug treatment [9,23,24,27,34,35,40,70]. Therefore, there has been a growing interest to find phytochemicals which would overcome the resistance to chemotherapeutic agents and consequently increase the efficacy of applied treatment [3,9,23,34,69,70]. Such combinations of dietary flavonoids with conventional antitumor drugs may lead to synergistic cytotoxic responses and offer a promising new strategy for cancer chemotherapy [5,30,34,69].

Cisplatin and paclitaxel are commonly used chemotherapeutic agents in the first-line therapy of ovarian cancer; however, both drugs can frequently induce complex and multifactorial resistance implying also that multiple cellular strategies can be employed to overcome it [3,5,33,84]. Therefore, combining agents with distinct molecular mechanisms of action represents a promising direction in enhancing antitumor activity [3,23,27,34,69]. Also, improving the cellular uptake of conventional drugs can sensitize the tumor cells to therapy as well as increasing the susceptibility of cancer cells to apoptosis is an efficient approach to cancer treatment [1,3,24]. Indeed, it is well known that flavonoids

can target various key elements in cellular signaling pathways associated with programmed cell death, including the modulation of expression and activity of B-cell lymphoma-2 (Bcl-2) family members as well as different caspases [85,86]. However, as every chemoresistant cell line has some phenotypic uniqueness the sensitizing efficacy of phytochemicals depends largely on the specific cells [33].

Data about the sensitization of ovarian cancer cell lines to chemotherapy drugs by natural flavonoids are compiled in Table 3. The main bioactive component of green tea, EGCG, has shown to potentiate the cytotoxicity of cisplatin both in chemosensitive ovarian cancer cell line A2780 as well as its several chemoresistant lines. Such combined treatment can increase the cellular accumulation of platinum and the platinum-DNA binding, weakening thus the effect of DNA repair. EGCG may also enhance the oxidative stress to suppress the growth of ovarian cancer cells and sensitize them to cisplatin [5,8,20,27]. However, the most efficient regimens of sequenced combinations of EGCG and cisplatin seem to depend on specific cell lines (Table 3).

The widely distributed dietary flavonol quercetin may also behave as a promising candidate for combined chemotherapy

Table 3: Sensitization of ovarian cancer cell lines to chemotherapy drugs by natural flavonoids.

Flavonoid	Cell line	Sensitized drug	Effect	Best regimen (h/h)	Reference
Flavanols					
EGCG	A2780, A2780^{cisR}	Cisplatin	Synergism; cellular accumulation of platinum and a high level of platinum DNA binding	0/4 (Cis/EGCG)	[5]
	A2780, A2780^{cisR}, A2780^{ZD0473R}	Cisplatin	Synergism	0/4 (Cis/EGCG)	[27]
	A2780/C200	Cisplatin	Increase in potency	0/24 (EGCG/Cis)	[20]
	CaOV3	Cisplatin	Increase in potency	0/24 (EGCG/Cis)	[20]
	SKOV3	Cisplatin	Increase in potency	0/24 (EGCG/Cis)	[20]
Flavones					
Protoapigenone	MDAH-2774	Cisplatin	Additive anticancer effect		[55]
Scutellarein	OVCAR3	Cisplatin	Synergism		[30]
Scutellarin	OVCAR3	Cisplatin	Synergism		[30]
Flavonols					
Kaempferol	OVCAR3	Cisplatin	Synergism; promotion of apoptosis		[84]
Quercetin	A2780, A2780^{cisR}	Cisplatin, oxaliplatin	Synergism	0/2 (Que/Cis or Que/Oxa)	[34]
	A2780P	Paclitaxel, cisplatin	Increase in sensitivity		[9]
	CaOV3	Cisplatin	Increase in sensitivity	0/24 (Que/Cis) or simultaneously	[65]
	EFO27	Paclitaxel	Increase in sensitivity		[9]
	OVCAR3	Paclitaxel, cisplatin	Increase in sensitivity		[9]
	OVCAR5	Tiazofurin	Synergism	0/12 (Tia/Que)	[87]
	SKOV3	Paclitaxel	Increase in sensitivity		[9]
		Cisplatin	Increase in sensitivity	0/24 (Que/Cis) or simultaneously	[65]
Isoflavones					
Genistein	A2780, A2780/C200	Cisplatin, taxotere, gemcitabine	Increase in inhibition and apoptosis		[28]
	A2780/CP70, 2008/C13	Cisplatin	Reversal of resistance; increase in cellular uptake of cisplatin		[33]
	UL-3C, UL-5, UL-6, UL-7, UL-8	Cisplatin, topotecan, paclitaxel	Increase in cytotoxicity		[14]
Tectorigenin	A2780^{TR}, MPSC1^{TR}, SKOV3^{TR} and their naive counterparts	Paclitaxel	Increase in potency; synergism in apoptosis		[3]
Flavanones					
Tangeretin	A2780/CP70, 2008/C13	Cisplatin	Synergism; increase in apoptosis	0/24 (Tan/Cis)	[69]
Flavanolignans					
Silibinin	A2780	Cisplatin	Potentiation of growth inhibition; prolonging cell cycle arrest in cisplatin-sensitive cells		[57]
	A2780/taxol	Paclitaxel	Reversal of resistance; enhancement of G2/M phase cell cycle arrest and apoptosis, reduction in invasiveness		[70]

against ovarian carcinoma, as this compound has described to be able to significantly increase the sensitivity of various ovarian cancer cells to different drugs (cisplatin, oxaliplatin, paclitaxel, and/or tiazofurin). Such potentiation is probably caused by targeting multiple pathways whereas the treatment outcome may be determined by the sequence of exposition of combined compounds [9,50,65,83,87]. Additionally, another common flavonol kaempferol can work synergistically with cisplatin in suppressing the growth of OVCAR3 ovarian cancer cells [84].

Isoflavone genistein supplementation could also sensitize different ovarian cancer cell lines, especially the drug resistant lines, to platinum and other conventional chemotherapeutic agents [14,28,33]. Furthermore, another natural isoflavone, tectorigenin is able to synergistically enhance the cytotoxic effect of paclitaxel in both chemoresistant ovarian tumor cell lines as well as their naive counterparts [3].

Treatment of malignant ovarian cells with citrus flavonoid tangeretin has shown to offer some possibilities for overcoming their resistance to cisplatin [69] and anticancer effect of this chemotherapeutic agent can also be enhanced by some flavones such as protoapigenone, scutellarein and scutellarin [30,55]. Likewise, flavanolignan silibinin is able to potentiate the cytotoxic activity of cisplatin in A2780 cells [57] and enhance the sensitivity of taxane-resistant subline to paclitaxel reducing also the invasiveness of tumor cells [70] (Table 3).

The increased sensitivity to chemotherapy induced by specific flavonoids in resistant ovarian cancer cell lines could be clinically relevant and provide therapeutic benefits [3,9,55,83,87]. The use of conventional chemotherapeutic drugs causes severe side effects and this toxicity poses the major limitation on their dosing [20,57]. Combination treatment of chemotherapy agents with specific dietary flavonoids may reduce the side effects of cytotoxic drugs because of lower doses are required to obtain an effective antitumor response [14,20,34,65,87,88]. Such combined drug regimen could thus be a promising new approach in treatment of human ovarian cancers and allows reducing the toxic side effects that patients have to suffer from chemotherapy treatment.

Conclusions

Ovarian carcinoma remains one of the most fatal female malignancies accounting for more deaths than any other gynecological cancers. Its treatment failure is often attributed to the resistance to conventional chemotherapeutic drugs and their toxic side effects and therefore, finding new compounds that are able to suppress ovarian cancer progression and target drug resistance is highly important for improving prognosis and increasing overall survival.

Natural products of plant origin are found to be interesting therapeutic agents for cancer prevention and treatment, whereas flavonoids may be a very promising class of phytochemicals exerting anticancer effects also in ovarian cancer cells. In this review article a contemporary overview of action of various natural flavonoids on different human ovarian tumor cell lines is presented showing the activity in both chemosensitive as well as resistant lines. Some polyphenolic compounds, such as wogonin and tectorigenin can exert even higher cytotoxicity to taxane-resistant sublines compared to their normal counterparts pointing to attractive therapeutic possibilities for treatment of chemoresistant tumors. However, the growth inhibitory effects of flavonoids are usually expressed in rather high concentration ranges (more than 20-30 μM); still, somewhat higher cytotoxicity has shown for green tea catechin EGCG, biflavone ginkgetin, flavone protoapigenone, and chalcone xanthohumol being active in low micromolar doses. The anticancer potency can be increased by synthetic approaches and in fact, by altering the chemical structure of isoflavone genistein, the new synthetic derivative phenoxodiol can exert even 30 times stronger efficacy in suppressing the viability of ovarian cancer cells compared to the naturally occurring lead compound [89,90]. The synthetic modification remains a perspective way to further enhance the anticancer activity of flavonoids in the future, maintaining their low toxicity to normal cells and improving also bioavailability.

Anticancer action of flavonoids is generally pleiotropic and the increase in cytotoxicity may occur through multiple pathways. Polyphenolic phytochemicals are able to inhibit cancer cell growth, trigger apoptosis, and induce cell cycle arrest in different phases depending on the specific structural features and also cell lines. In addition, some flavonoids such as apigenin and genistein are able to inhibit the invasion and migration of ovarian cancer cells retarding in this way the metastatic progression of tumors. The anticancer effect can be exerted also via suppressing the expression of VEGF, like in the case of kaempferol. As apoptotic pathways are commonly dysregulated in chemoresistant cell lines, circumvention of these cellular signaling alterations as well as executing the cell death through alternative non-apoptotic mechanisms are very important for effective treatment. Indeed, besides triggering apoptosis natural isoflavone genistein is also capable to induce autophagic cell death in cancerous ovarian cells. Also, it is possible that the anticancer activity of flavonoids may be mediated, at least in part, by the estrogen receptors as about two-thirds of ovarian epithelial carcinomas are estrogen receptor positive. In this context the variations in expression level of different subtypes (ERα and ERβ, but also type II EBS) can play an important role in determining the inhibitory activity of phytoestrogenic flavonoids.

The principal cause of high mortality and treatment failure of ovarian tumors involves the resistance of neoplastic cells to conventional chemotherapies. Combination treatment of these drugs with specific flavonoids (such as EGCG, quercetin, genistein) may lead to sensitization of cancerous cells to cytotoxic agents allowing for reducing their doses that are needed to obtain effective anticancer responses. Lowering the concentrations of chemotherapeutic drugs should ultimately decrease the systemic toxicity. Therefore, such combination strategies may have clinical benefits and certainly deserve further studies for possible therapeutic applications to improve the prognosis and survival of ovarian cancer patients.

More than 5000 different naturally occurring flavonoids have been described displaying a huge amount of structural variations. Studies of the action modes of these compounds, both alone as

well as in combination with conventional chemotherapy drugs, can open new perspectives in the management of ovarian cancer and may improve the treatment outcome of this devastating disorder.

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