REVIEW

Characteristic Features of Cytotoxic Activity of Flavonoids on Human Cervical Cancer Cells

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Abstract

Cervical cancer is the most common gynecologic malignancy worldwide and development of new therapeutic strategies and anticancer agents is an urgent priority. Plants have remained an important source in the search for novel cytotoxic compounds and several polyphenolic flavonoids possess antitumor properties. In this review article, data about potential anticarcinogenic activity of common natural flavonoids on various human cervical cancer cell lines are compiled and analyzed showing perspectives for the use of these secondary metabolites in the treatment of cervical carcinoma as well as in the development of novel chemotherapeutic drugs. Such anticancer effects of flavonoids seem to differentially depend on the cellular type and origin of cervical carcinoma creating possibilities for specific targeting in the future. Besides the cytotoxic activity per se, several flavonoids can also contribute to the increase in efficacy of conventional therapies rendering tumor cells more sensitive to standard chemotherapeutics and irradiation. Although the current knowledge is still rather scarce and further studies are certainly needed, it is clear that natural flavonoids may have a great potential to benefit cervical cancer patients.

Keywords: Cervical cancer - natural flavonoids - anticancer mechanisms - chemosensitization

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Introduction

Cervical cancer is the term for malignant neoplasm arising from cells originating from the cervix uteri (Liu et al., 2012; Jin et al., 2014). It is a common malignancy of women remaining one of the leading causes of cancer-related death worldwide (Hussain et al., 2012; Lo et al., 2012; Zeng et al., 2012a; Chen YJ et al., 2013a; Chou et al., 2013; Dayuthapani et al., 2013; Lo et al., 2013; Jin et al., 2014). According to the GLOBOCAN 2012 data, cervical cancer is the fourth most common cancer among women in terms of both incidence and mortality rates worldwide, with an estimated annual incidence of 528 000 new cases and 266 000 deaths in 2012 (Globocan2012). Accounting for 7.5% of all female cancer deaths it is the most common gynecologic malignancy and the most frequent cause of gynecologic cancer deaths globally (Singh et al., 2010; Globocan2012; Muthusami et al., 2013; Wang et al., 2013; Garcia et al., 2014).

In spite of the screening methods and early detection programs, invasive cervical cancer still represents a major concern for public health (Zou et al., 2010; Di Domenico et al., 2012; Hussain et al., 2012; Tudoran et al., 2012). Whereas both the incidence and mortality of cervical cancer have decreased in developed countries, more than 80% of the new cases occur in the developing countries being diagnosed mostly in the advanced stages and impacting the lives of women during their years of the highest productivity (Singh et al., 2010; Zou et al., 2010; Tudoran et al., 2012; Zhang et al., 2012; Kitdamrongtham et al., 2013; Zhu et al., 2013). Thus, a woman`s risk of developing cervical cancer by 65 years of age has been estimated to range from 0.69% in developed countries to 1.38% in developing countries (Muthusami et al., 2013).

The major etiological factor in the formation of cervical cancer is the infection with human papillomavirus (HPV) and more than 99% of cervical tumors contain one or more of the oncogenic HPV genotypes (Lee et al., 2011; Di Domenico et al., 2012; Kim et al., 2012; Alshatwi et al., 2013; Kim MS et al., 2013; Zhu et al., 2013; Garcia et al., 2014; Ham et al., 2014). There are more than 200 types of HPVs described to date from which about 15 are designed as high-risk strains according to the propensity of malignant progression of virus-associated lesions (Di Domenico et al., 2012; Yuan CH, 2012; Hirchaud et al., 2013; Moga et al., 2014). The most prevalent high risk type is HPV16 that is responsible for the development of about 50-60% of all cervical cancers. It is followed by HPV18 that harbors about 10-20% of cervical carcinomas worldwide (Alshatwi et al., 2013; Cherry et al., 2013; Hirchaud et al., 2013; Kim MS et al., 2013). The oncogenic function of HPVs can be primarily attributed to the two encoded oncoproteins, E6 and E7 (Kim et al., 2012; Kim MS et al., 2013). The E6 protein target the wild-type tumor suppressor p53, binding to it and stimulating its degradation; the E7 protein interacts to the other tumor suppressor, retinoblastoma (Rb). These two oncoproteins induce cervical cancer progression by disturbing the

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functions of tumor suppressors on cell cycle regulation and apoptosis, thereby enhancing the proliferation of infected cells and promoting their immortality (Lee et al., 2005; Shin et al., 2008; Lee et al., 2011; Di Domenico et al., 2012; Kim et al., 2012; Hirchaud et al., 2013; Kim MS et al., 2013). Downregulation of these oncoproteins could thus enhance the stabilization and increase the level of tumor suppressors, engaging cancer cells in cell death.

Although the persistent infection with oncogenic HPV type is necessary to cervical carcinogenesis other factors are also required for the malignant progression. Such cofactors include cigarette smoking, chronic inflammations, infections with *Chlamydia trachomatis* and herpes simplex virus-2, but also being the daughter of a woman who used the drug diethylstilbestrol during pregnancy to prevent miscarriage (Di Domenico et al., 2012; Zeng et al., 2012b; Alshatwi et al., 2013; Jin et al., 2014). Moreover, there are some evidences that estrogen may contribute to the genesis of cervical cancer by increasing the proliferation of HPV-infected cells and promoting the expression of oncoproteins E6/E7 (Hernandez et al., 2004; Qiao et al., 2009).

To date, there are no antiviral treatments for HPV infection but the prophylactic vaccination is an effective primary prevention strategy for cervical malignancies (Cherry et al., 2013; Garcia et al., 2014). Nonetheless, the current vaccines are restricted only to the applications for two oncogenic strains (16 and 18) leaving nearly a third of the high-risk HPV types without a primary prevention strategy. Also, these vaccines offer no benefit for the already infected people and because of the high cost they are rather inaccessible to the populations in developing countries where the incidence of cervical cancer is highest (Yuan CH, 2012; Wang et al., 2013; Zhu et al., 2013; Garcia et al., 2014). For these reasons, studies of novel therapeutic strategies for management of already existing preinvasive and invasive lesions are highly important in the fight against cervical cancer.

If detected early, cervical cancer is generally curable; however, treatment of metastatic or recurrent carcinoma is poorly effective and with serious side effects (Tudoran et al., 2012; Muthusami et al., 2013). Conventional therapies include surgery, radiotherapy, chemotherapy, and immunotherapy (Liu et al., 2012; Chen et al., 2013b). Radical surgery or radiotherapy can be curative for the majority of patients with early-stage cervical cancer; however, chemotherapy is always the first choice for patients with the advanced disease for which the prognosis remains very poor (Zhang B et al., 2006; Li et al., 2009; Kim et al., 2010; Al-Hazzani and Alshatwi, 2011; Ju et al., 2012; Alshatwi et al., 2013; Ramesh and Alshatwi, 2013; Zhu et al., 2013; Jin et al., 2014). Although the platinum- or taxane-based chemotherapy either neoadjuvant before surgery or combined with radiotherapy can significantly improve the survival of patients, the success of these treatment modalities is often hindered by emerging of chemoresistance and/or radioresistance as well as by severe lethality on normal cells resulting in serious toxicity (Hsu et al., 2009; Koppikas et al., 2010; Singh et al., 2010; Liu et al., 2012; Chen et al., 2013b; Chen et al., 2013a; Lo et al., 2013; Singh et al., 2013; Zhu et al., 2013). Therefore, the survival benefit of conventional therapies is limited, relapse can occur after treatment, and cervical cancer continues to have one of the lowest five-year survival rates being only about 52% (Hsu et al., 2009; Singh et al., 2010; Chen et al., 2011; Zeng et al., 2012a; Kim and Kim, 2013; Singh et al., 2013). Consequently, the development of more effective, highly selective and less toxic anticancer agents as well as novel therapeutic intervention strategies are needed to improve the treatment success and reduce the cervical cancer morbidity and mortality rate.

Natural Flavonoids as a Potential Source for Novel Chemotherapeutics

Numerous effective anticancer drugs are either isolated from botanical sources or are modifications of natural molecules (Csapi et al., 2010; Al-Hazzani and Alshatwi, 2011; Hirchaud et al., 2013; Kitdamrongtham et al., 2013; Muthusami et al., 2013). Plants have received extensive attention as a source of potential antitumor agents in the past few decades and currently, most of the modern anticancer drugs such as camptothecin, vincristine, vinblastine, adriamycin, taxol, etoposide and paclitaxel are plant-derived compounds (Koppikas et al., 2010; Vidya Priyadarsini et al., 2010; Krifa et al., 2013). Due to a significant untapped resource probably still remained in herbal medicines, it is crucial to keep up the search for plants with antitumor activity providing promising new leads for development of novel chemotherapeutics (Csapi et al., 2010; Al-Hazzani and Alshatwi, 2011; Ju et al., 2012; Meiyanto et al., 2012; Alonso-Castro et al., 2013).

Fruits, vegetables, some common beverages, and several medicinal herbs are rich sources of minor non-nutritive constituents with potential tumoricidal properties (Bhatia et al., 1999; Cherng et al., 2007; Hussain et al., 2012; Kma, 2013; Kundu and Chun, 2014). These secondary metabolites can act on almost all stages of carcinogenesis including initiation, promotion and progression, and may also interfere with chemo- and radiotherapy in patients undergoing cancer treatment (Di Domenico et al., 2012; Hussain et al., 2012; Sharma et al., 2012). Besides the diverse and complex biological effects such dietary compounds are generally relatively safe exerting lower toxicity and fewer side effects than traditional chemotherapeutic agents (Tudoran et al., 2012; Kim and Kim., 2013; Muthusami et al., 2013).

In particular, flavonoids as the ubiquitous polyphenolic phytochemicals are known to have cytotoxic and antitumor effects and are intensely studied in recent years (Ju et al., 2012; Ying et al., 2012; Chen et al., 2013b). Flavonoids constitute a class of more than 4000 phenylbenzopyrones and even minor modifications in their structure can be responsible for strong variations in the spectrum of biological activities (Cherng et al., 2007; Mamadalieva et al., 2011). These compounds possess multiple anticancer effects including antioxidant activity, antiproliferation, cell cycle arrest, promotion of apoptosis, modulation of signal transduction pathways and regulation of hormone receptors, inhibition of cancer cell migration, invasion and angiogenesis, and reversal of multidrug resistance (Totta et al., 2004; Kanno et al., 2005; Yang et al., 2009; Chen et

al., 2013a; Ramesh and Alshatwi, 2013; Deng et al., 2014; Hu et al., 2014). The selectivity of flavonoids on their molecular targets is probably much higher than previously expected making these compounds very interesting lead structures for the development of novel chemotherapeutic agents (Mamadalieva et al., 2011). However, the systemic bioavailability of these compounds is reduced by metabolic conjugation in gastrointestinal tract and liver and only a very small amount of these polyphenols enters the plasma in unchanged form. The absorption from gut depends on the molecular structure, consumed amount, degree of bioconversion, and nutrient status and genetic factors of the host, so that the bioavailability and intestinal metabolism of flavonoids can be markedly different from one another (Thomasset et al., 2006; Tumbas et al., 2012; Ramesh and Alshatwi, 2013).

Cervical cancer develops through a multistep carcinogenesis and has a long premalignant phase termed cervical intraepithelial neoplasia (Zou et al., 2010; Di Domenico et al., 2012). Such protracted preinvasive character provides opportunities for an effective medical treatment and interfering also with dietary agents (Cherry et al., 2013; Garcia et al., 2014). Epidemiologic studies have indeed suggested that modifying the diet can reduce the risk of cervical cancer being related to the susceptibility to and/or persistence of HPV infection (Sah et al., 2004; Butler and Wu, 2011; Di Domenico et al., 2012; Ramesh and Alshatwi, 2013). However, there are still rather little known regarding the effects of polyphenolic phytochemicals in cervical carcinogenesis (Hernandez et al., 2004; Singh et al., 2010) and therefore, this comprehensive study was undertaken to compile and analyze the data about the effects of most common natural flavonoids on various human cervical cancer cell lines. Especially in low-resource settings, the use of inexpensive dietary intervention would be an attractive adjunct to cervical cancer prevention strategies (Zou et al., 2010).

Human Cervical Cancer Cell Lines Used for Studies of Flavonoids Anticancer Effects

Several human cervical cancer cell lines have been used to study the potential anticancer action of flavonoids, differing from one another by origin as well as the HPV status. As the oncogenic types HPV16 and HPV18 are responsible for approximately 70% of all cervical cancer cases worldwide (Lee et al., 2011), most of the *in vitro* experiments are also performed by using human cervical cancer cell lines positive for HPV16 or HPV18.

The HPV18 type HeLa cells are widely studied adenocarcinoma line expressing low level wild type p53 (see Table 1). Also, the other HPV18-positive lines (TMCC-1 and KB-V1/Vbl) are derived from cervical adenocarcinoma tissues. On the other hand, the origin of HPV16-positive lines CaSki and SiHa is squamous cell carcinoma. Adenocarcinoma and squamous cell carcinoma are two main types of cervical cancers. Whereas adenocarcinomas of the uterine cervix represent only 15-20% of all primary cervical carcinomas their incidence has grown increasingly during the past decades. Moreover, cervical adenocarcinomas are relatively more aggressive, tend to metastasize earlier and are less sensitive to chemo- and radiotherapy than their squamous cell counterparts. Also, their prognosis is less favorable exerting somewhat lower five-year survival rate than that of squamous cell carcinomas (Noguchi et al., 2006; Zhang B et al., 2006; Yokoyama et al., 2008; Al-Hazzani and Alshatwi, 2011). Therefore, the development of new anticancer drugs for the treatment of adenocarcinomas is an especially important task.

Both CaSki and SiHa human cervical cancer cells express oncogenic type HPV16. Whereas the former line contains 60-600 copies of virus genome per cell and continuously expresses the HPV E6 oncoprotein, the SiHa cells contain only 1 or 2 copies of integrated HPV16 DNA per cell (Table 1).

Studies of the flavonoids on cervical cancer cell lines immortalized with other oncogenic HPV types are much more limited, as only the data about HPV68-positive ME180 cells derived from squamous cell carcinoma of uteri cervix can be found. This leaves the issue of potential HPV-subtype-specific effects rather obscure. Nonetheless, two HPV-free cell lines, C33A and OMC-4 are still included in the studies, both expressing mutated p53 (Table 1).

Anticancer Action of Natural Flavonoids on Cervical Cancer Cell Lines

In this work, data about the anticancer effects of different types of natural flavonoids (chalcones, flavanols, flavanones, flavones, flavonols, and isoflavones) on various human cervical cancer cell lines were compiled from the literature and analyzed according to the potency and action modes of cytotoxic activity. These data are summarized in the Table 2 and discussed systematically below.

Chalcones

Chalcones are naturally occurring flavonoids, whereas several such compounds have shown to represent promising tools for cancer treatment (Bazzaro et al., 2011).

The common dietary licorice chalcone isoliquiritigenin has shown to reduce the cell viability by inducing cell cycle arrest and morphological and biochemical features of apoptosis in both HPV16- (CaSki, SiHa) and HPV18-positive (HeLa) cell lines as well as HPV-negative cervical cancer cells (C33A) (Li et al., 2008; Hsu et al., 2009; Park et al., 2009; Hirchaud et al., 2013). Increase in intracellular reactive oxygen species (ROS) and triggering oxidative stress may play a critical role in isoliquiritigenin induced HeLa cell apoptosis (Yuan X et al., 2012; Yuan et al., 2013). In CaSki cells, this chalcone downregulates the HPV16 oncoprotein E6 expression followed by a subsequent increase in the level of p53 tumor suppressor and promoting the cancer cell death (Hirchaud et al., 2013). The somewhat higher cytotoxic potency of isoliquiritigenin in CaSki cells compared to that of SiHa cells (see Table 2) can be associated with harboring more copies of integrated HPV16 genome. Indeed, whereas CaSki cells contain up to 600 copies of HPV16 DNA per cell, there are only 1 or 2 copies of integrated HPV16

Table 1. Characteristics of Human Cervical Cancer Cell Lines

Cell line	HPV status	Further description	References
C33A	Negative	p53 mutated	Lee et al., 2005; Hirchaud et al., 2013; Kim MS et al., 2013
OMC-4	Negative	Well-differentiated adenocarcinoma cell line; p53 mutated	Noguchi et al., 2006
CaSki	HPV16	Squamous cancer cell line derived from a metastatic epidermoid cervical cancer; contains 60-600 copies per cell of integrated HPV16 genome; p53 wild type	Ahn et al., 2003a; Lee et al., 2005; Shin et al., 2008; Lee et al., 2011; Cherry et al., 2013; Hirchaud et al., 2013; Kim MS et al., 2013
SiHa	HPV16	Squamous carcinoma cell line; contains only 1 or 2 copies per cell of HPV16 genome; p53 wild type; resistant to cisplatin	Yokoyama et al., 2004; Lee et al., 2005; Singh et al., 2010; Lee et al., 2011; Di Domenico et al., 2012; Kim MS et al., 2013
HeLa	HPV18	Epithelial adenocarcinoma cell line; contains 10-50 copies per cell of HPV18 genome; p53 wild type; absence of any estrogen receptor isoforms	Wang et al., 2001; Samama et al., 2002; Guo et al., 2004; Totta et al., 2004; Virgili et al., 2004; Zhang B et al., 2006; Hirchaud et al., 2013
KB-V1/Vbl	HPV18	Adenocarcinoma cell line; multidrug-resistant	Pluchino et al., 2012
TMCC-1	HPV18	Poorly differentiated adenocarcinoma cell line	Yokoyama et al., 2004; Noguchi et al., 2006; Yokoyama et al., 2008
ME180	HPV68	Squamous carcinoma cell line; p53 wild type; absence of any estrogen receptor isoforms	Yokoyama et al., 2004; Wang et al., 2001

genome in SiHa cells (Table 1).

Flavanols

Flavanols are a major component of green tea and epigallocatechin-3-gallate (EGCG) is the most abundant and bioactive polyphenol in green tea (Di Domenico et al., 2012).

There is ample evidence about the effects of EGCG on both inhibiting growth and promoting apoptosis in HPV-infected cervical cancer cells and cervical cancer cell lines. The anticancer action of EGCG includes numerous mechanisms and occurs via various pathways depending on the nature and origin of the cells (Sah et al., 2004; Butler and Wu, 2011; Muthusami et al., 2013). Thus, the antiproliferative activity of EGCG is described in both HPV-positive as well as HPV-negative human cervical cancer cell lines (see Table 2) and this polyphenolic compound could induce the cell cycle arrest and/or promote apoptosis in HPV16-associated CaSki cells (Ahn et al., 2003a; Qiao et al., 2009; Di Domenico et al., 2012), HPV18-positive HeLa and TMCC-1 cells (Borska et al., 2003; Yang et al., 2003; Noguchi et al., 2006; Qiao et al., 2009; Sharma et al., 2012; Muthusami et al., 2013) but also in HPV-negative OMC-4 cells (Noguchi et al., 2006). In CaSki and HeLa cells EGCG can cause reduction in oncoproteins E6 and E7 expression leading to increase in p53 protein and suppression of cancer cell growth (Qiao et al., 2009; Zou et al., 2010). Moreover, EGCG is also able to effectively inhibit the invasion and migration of HeLa cells by modulating the expression of related genes and impeding the metastasis process (Zhang Q et al., 2006; Sharma et al., 2012; Tudoran et al., 2012).

As a characteristic feature, the generally lower cytotoxic activity of EGCG in cervical adenocarcinoma cell lines compared to their squamous cell counterparts is reported (Noguchi et al., 2006; Yokoyama et al., 2008). In this way, EGCG can express more effective growth inhibitory activity in squamous cell carcinoma cell lines SiHa and ME180 than in adenocarcinoma lines TMCC-1 and HeLa (Yokoyama et al., 2004; Zou et al., 2010) and CaSki cells reveal higher sensitivity to EGCG than HeLa cells (Qiao et al., 2009). These data suggest that the anticancer effects of EGCG may differentially depend on the cellular type and origin of cervical carcinoma.

On the other hand, cervical adenocarcinoma cell line TMCC-1 seems to be less sensitive to EGCG exposure compared to the another adenocarcinoma line HeLa (Yokoyama et al., 2008) possibly indicating the differences in copies of integrated HPV18 genome per cells.

The potency of anticancer action of EGCG in human cervical cells is dependent also on the grade of malignancy as this dietary flavanol is in general more effective in premalignant cells and the sensitivity to EGCG decreases with the progression of carcinogenic process (Yokoyama et al., 2004; Noguchi et al., 2006; Yokoyama et al., 2008). At the same time the cytotoxic activity of EGCG on normal cervical cells is much lower than on the respective cancerous cells (Yang et al., 2003; Yokoyama et al., 2004).

Considering the aforementioned knowledge and the relatively low toxicity and effective oral bioavailability of green tea constituent EGCG, it is evident that this compound holds a great potential in the treatment of cervical cancer, both from the preventive as well as therapeutic aspects, and EGCG could be regarded as a promising candidate for the development of novel anticancer drugs (Ahn et al., 2003b; Di Domenico et al., 2012; Sharma et al., 2012; Muthusami et al., 2013).

Flavanones

Although the treatment of human cervical cancer cells (positive for HPV16 or HPV18) with widespread dietary flavanones including fruit flavonoids hesperetin, naringenin and naringin can lead to a dose- and time-dependent inhibition of proliferation (see Table 2), this activity reveals only at rather high concentrations (usually more than 100μM) and there are no cytotoxic effects on the cell viability at lower doses (Virgili et al., 2004; Kanno et al., 2005; Liu et al., 2011; Kim et al., 2012; Xie SR et al., 2012; Alshatwi et al., 2013; Ramesh and Alshatwi, 2013; Deng et al., 2014). This anticancer action occurs

Table 2. Cytotoxic Activity of Flavonoids on Human Cervical Cancer Cell Lines

Flavonoid/Cell line	IC_{50}, µM (mean)	Assay method*	Incubation h	Cytotoxic action	References
Chalcone					
Isoliquiritigenin					
C33A	32.8	MTT	72	Induction of G2/M phase cell cycle arrest and apoptosis	Hirchaud et al., 2013
CaSki	39.1	MTT	72	Induction of G2/M or G0/G1 phase cell cycle arrest and apoptosis; decrease in HPV16 E6 expression	Hirchaud et al., 2013
SiHa	53.8	MTT	72	Induction of G2/M phase cell cycle arrest and apoptosis	Hirchaud et al., 2013
HeLa	40	SRB	24	Induction of apoptosis by increase in ROS levels	Yuan X et al., 2012
	9.8	XTT	48	Induction of G2/M phase cell cycle arrest and apoptosis	Hsu et al., 2009
	58.1-82.6 (70.4)	MTT	72	Induction of G2/M phase cell cycle arrest and apoptosis	Li et al., 2008; Hirchaud et al., 2013
Flavanol					
EGCG OMC-4	~100	Cell counts	48	Induction of apoptosis	Noguchi et al., 2006
CaSki	27.3-35 (31.1)	MTT; cell counts	24	Induction of G2/M or G1 phase cell cycle arrest and apoptosis; inhibition of E6 and E7 expression	Ahn et al., 2003a; Qiao et al., 2009
HeLa	47.9-100 (74.0)	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis; inhibition of E6 and E7 expression; suppression of cell invasion and migration	Qiao et al., 2009; Sharma et al., 2012; Muthusami et al., 2013
	20.4-66 (45.5)	MTT; cell counts	48	Induction of apoptosis; inhibition of cell invasion and migration	Borska et al., 2003; Yang et al., 2003; Sharma et al., 2012; Tudoran et al., 2012; Muthusami et al., 2013
TMCC-1	~100	Cell counts	48	Induction of apoptosis	Noguchi et al., 2006
Flavanones					
Hesperetin SiHa	650	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis	Alshatwi et al., 2013
Liquiritigenin					
HeLa	247	MTT	48	Induction of apoptosis	Liu et al., 2011
	137 (>100)	MTT	72	Induction of apoptosis	Li et al., 2008; Liu et al., 2011
Naringenin					
CaSki	NE up to 15µM	MTS	24, 48		Kim et al., 2012
SiHa	NE up to 15µM	MTS	24, 48		Kim et al., 2012
HeLa	223-243 (233)	MTT	48		Kanno et al., 2005; Deng et al., 2014
	>100	MTT	72		Li et al., 2008; Li et al., 2010
Naringin SiHa	750	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis	Ramesh and Alshatwi, 2013
Pinocembrin					
HeLa	428.6	MTT	24		Mamadalieva et al., 2011
	>100	MTT	72		Li et al., 2010
Flavones					
Amentoflavone					
C33A	NE up to 100µM	MTS	24, 48		Lee et al., 2011
CaSki	Effect at >20µM	MTS	24, 48	Inhibition of E7 protein expression	Lee et al., 2011
SiHa	Effect at >40µM	MTS	24, 48	Inhibition of E7 protein expression	Lee et al., 2011
Apigenin					
CaSki	NE up to 20µM	MTT	24	Inhibition of invasion and migration	Noh et al., 2010
SiHa	WE up to 44.4µM	MTS	24		Wu et al., 2006
HeLa	123.2	MTT	24		Mamadalieva et al., 2011
	6-85.3 (19.5)	XTT; MTT; cell counts	72	Induction of G1 phase cell cycle arrest and apoptosis	O`Prey et al., 2003; Zheng et al., 2005; Kuo et al., 2008; Csupor-Löftler et al., 2009; 2011; Csapi et al., 2010; Li et al., 2010
KB-V1/Vbl	20.6	MTT	72		Spoerlein et al., 2013
Apigenin-7-O-glucoside					
HeLa	318.8	MTT	24		Mamadalieva et al., 2011
Chrysin HeLa	89.7	MTT	24		Mamadalieva et al., 2011
	13-69.5 (32.2)	MTT; hexosaminidase	72	Induction of apoptosis	Zhang et al., 2004; Cardenas et al., 2006; Li et al., 2010
KV-V1/Vbl	12.5	MTT	72		Spoerlein et al., 2013
Eupafolin HeLa	26.8	MTT	48	Induction of apoptosis	Chung et al., 2010
Eupatorin HeLa	29.8	MTT	72		Csapi et al., 2010
Hispidulin HeLa	34.6	MTT	72		Xu et al., 2013
Hispidulin 7-O-b-D-glucopyranoside					
HeLa	>108.2	MTT	72		Xu et al., 2013
7-Hydroxyflavone					
HeLa	51.9	MTS	24	Induction of G0/G1 phase cell cycle arrest and apoptosis	Zhang T et al., 2012
	32.1	MTS	72	Induction of G0/G1 phase cell cycle arrest and apoptosis	Zhang T et al., 2012
Jaceosidin C33A	NE up to 30.3µM	WST-1	20		Lee et al., 2005
CaSki	Effect at >15µM	WST-1	20	Inhibition of E6 and E7 oncoproteins	Lee et al., 2005
SiHa	Effect at >15µM	WST-1	20	Inhibition of E6 and E7 oncoproteins	Lee et al., 2005
HeLa	NE up to 30.3µM	WST-1	20		Lee et al., 2005
	46.3	MTT	72		Xu et al., 2013
Jaceosidin 7-O-b-D-glucopyranoside					
HeLa	>101.6	MTT	72		Xu et al., 2013
Luteolin C33A	NE up to 20µM	MTS	24, 48		Ham et al., 2014
CaSki	WE at >40µM	MTS	24, 48		Ham et al., 2014
SiHa	WE at >40µM	MTS	24, 48		Wu et al., 2006; Ham et al., 2014
HeLa	21.8	MTT	24	Induction of G2/M cell cycle arrest and apoptosis	Krifa et al., 2013
	Effect at >5µM	MTS	48	Induction of apoptosis; suppression of E6 and E7 levels	Ham et al., 2014
	5.8-7.6 (6.7)	MTS; MTT; cell counts	72		O`Prey J et al., 2003; Csupor-Löftler et al., 2009; Cherry et al., 2013
Morusin HeLa	0.94	MTT	72		Wang et al., 2013
Oroxylin A HeLa	19.4	MTT	48	Induction of apoptosis	Li et al., 2009
Wogonin C33A	NE up to 160µM	MTS	48		Kim MS et al., 2013
CaSki	Effect at >40µM	MTS	48	Induction of apoptosis; inhibition of E6 and E7 expression	Kim et al., 2013
SiHa	Effect at >40µM	MTS	48	Induction of apoptosis; inhibition of E6 and E7 expression	Kim et al., 2013
HeLa	Effect at >30µM	MTT	24	Induction of G1 phase cell cycle arrest and apoptosis	Yang et al., 2009
	Effect at >30µM	MTT	48	Induction of G1 phase cell cycle arrest and apoptosis	Yang et al., 2009

Table 2 (Continued). Cytotoxic Activity of Flavonoids on Human Cervical Cancer Cell Lines

Flavonols					
Fisetin CaSki	NE up to 40μM	MTT	24, 48	Decrease in cell motility; inhibition of migration/invasion	Chou et al., 2013
SiHa	NE up to 40μM	MTT	24, 48	Decrease in cell motility; inhibition of migration/invasion	Chou et al., 2013
HeLa	52.0	MTT	24	Induction of apoptosis	Ying et al., 2012
	36.0	MTT	48	Induction of apoptosis	Ying et al., 2012
Galangin HeLa	43.6	MTT	72		Li et al., 2010
Kaempferol HeLa	13-23.1 (17.7)	MTT; cell counts	72		O`Prey et al., 2003; Kuo et al., 2008; Dat et al., 2010
Kaempferol 7-O-b-D-glucoside					
HeLa	93.9	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis	Xu et al., 2008
	44.8	MTT	36	Induction of G2/M phase cell cycle arrest and apoptosis	Xu et al., 2008
Kaempferitrin					
HeLa	45	MTT	48	Induction of G1 phase cell cycle arrest and apoptosis; triggering ROS generation	Alonso-Castro et al., 2013
Quercetin HeLa	110.4	MTT	18	Induction of G2/M phase cell cycle arrest and apoptosis; triggering ROS generation; inhibition of migration	Bishayee et al., 2013
	80	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis	Vidya Priyadarsini et al., 2010
	34.1	MTT; SRB	48		Tumbas et al., 2012; Deng et al., 2014
	5	MTT; cell counts	72	Induction of apoptosis	O`Prey et al., 2003; Jung et al., 2010
Isoflavone					
Genistein C33A	Effect at >40μM	MTT	48	Induction of apoptosis	Kim et al., 2009b
CaSki	60	MTT	48	Induction of apoptosis	Kim et al., 2009a; Kim et al., 2009b
SiHa	80	MTT	48	Induction of apoptosis	Jha et al., 2010
HeLa	126	MTT	24	Induction of G2/M phase cell cycle arrest and apoptosis	Zhou et al., 2009
	20-100 (57.5)	MTT; cell counts	48	Induction of G2/M or S phase cell cycle arrest and apoptosis; inhibition of migration/invasion	Wang et al., 2001; Kim et al., 2009a; Kim et al., 2009b; Zhou et al., 2009; Hussain et al., 2012
KB-V1/Vbl	28.8	MTT	72		Spoerlein et al., 2013
ME180	60	Cell counts	48	Induction of G2/M phase cell cycle arrest and apoptosis; inhibition of invasion	Wang et al., 2001

*Cell viability evaluation methods: MTT-assay, MTS-assay, XTT-assay, SRB (sulforhodamine B)-assay, WST-1 assay, counting of viable cells using hemocytometer or plate counter, colorimetric determination of hexosaminidase level; NE, no effect; WE, weak effect

through induction of cell cycle arrest and promotion of apoptosis. Moreover, liquiritigenin is also able to inhibit the expression of vascular endothelial growth factor (VEGF) and interfere with tumor angiogenesis, thereby suppressing the progression of cervical cancer (Liu et al., 2012; Xie SR et al., 2012).

Flavones

Several natural flavones exhibit dose- and time-dependent cytotoxic activity in different human cervical cancer cell lines already at low micromolar concentrations (see Table 2). These antitumorigenic properties can be mediated through versatile biological mechanisms, including alteration of cell cycle progression, induction of morphological and biochemical changes characteristic of apoptosis, and suppression of expression of E6/E7 oncoproteins. Furthermore, apigenin is also able to inhibit cell motility and reduce invasive potential of cervical adenocarcinoma HeLa cells and squamous cell carcinoma CaSki cells suggesting that this widespread dietary compound may exert its anticancer effect *in vivo* via suppression of tumor cell penetration of the healthy tissues. Therefore, treatment with apigenin might be a useful therapeutic and/or adjuvant therapeutic strategy for controlling metastasis and invasiveness of cervical tumors leaving this flavone a powerful candidate in developing of new antimetastatic agents (Czyz et al., 2005; Noh et al., 2010).

One of the most potent and interesting natural flavones described so far is morusin, with the half-maximal cytotoxic concentration in HeLa cells of only 940 nM. This flavone derivative can inhibit the growth and migration and induce apoptosis even of human cervical cancer stem cells deserving certainly further investigation as a novel therapeutic drug (Wang et al., 2013).

In addition, the data compiled in Table 2 provide some hints about the stronger inhibitory potency of flavone aglycones compared to the respective glycosides. In this way, apigenin, hispidulin and jaceosidin are all about 3 times more cytotoxically active than their sugar derivatives in HeLa cells.

As an interesting and perspective phenomenon the HPV-infection-specific and even HPV-subtype-specific anticancer action is described for some flavones. First, the biflavone amentoflavone is shown to inhibit the proliferation and induce apoptotic death in HPV16-positive cervical cancer cells CaSki and SiHa, but does not exert any significant cytotoxic effect on the growth of HPV-non-harboring C33A cells (Lee et al., 2011). Some differences in the sensitivity of this biflavone to CaSki and SiHa cells are probably associated with variations in the copy numbers of integrated HPV16 genome, as CaSki cells contain up to 600 copies of virus genome compared to only one or two copies of HPV16 DNA in SiHa cells (Lee et al., 2011). Second, also wogonin is selectively effective against human cervical cancer cells harboring HPV genome: it is cytotoxic in cervical cancer cells containing DNA for HPV16 (SiHa, CaSki) or HPV18 (HeLa) giving no decrease in the viability of HPV-negative cells (C33A) (Yang et al., 2009; Yang et al., 2011; Kim MS et al., 2013). These data suggest that in the HPV-infected cells the apoptotic death is promoted by suppressing the expression of E6 and E7 oncoproteins and restoring the p53 and pRb pathways.

Furthermore, jaceosidin is proved to specifically inhibit HPV16-harboring cervical carcinoma cell lines CaSki and SiHa and exert only very little or no inhibition in HPV18-positive HeLa cells and HPV-negative C33A cells (Lee et al., 2005). On the contrary, luteolin significantly inhibits the proliferation and induces

apoptosis of HPV18-positive HeLa cells exhibiting only mild cytotoxicity at high doses in HPV16-harboring cells and no effect on HPV-free C33A cells (Xie F et al., 2012; Krifa et al., 2013; Ham et al., 2014). It is probable that jaceosidin can specifically downregulate the E6 and E7 oncoproteins of HPV type 16 and luteolin those of HPV type 18. Such specific targeting provides an alternative approach for prevention and treatment of preinvasive and invasive lesions of uterine cervix related to infection with certain papillomavirus types and gives thus potential for development of new therapeutic agents for human cervical cancer. The specific effect of luteolin on HPV18-positive human adenocarcinoma HeLa cells might be particularly interesting as adenocarcinoma of the uterine cervix is known to be less-responsive to radiation therapy and currently used chemotherapeutics (Noguchi et al., 2006).

Flavonols

A number of well-known and widespread natural flavonols are shown to exhibit anticancer effects in human cervical cancer cells by inducing cell cycle arrest in different phases (G1 or G2/M) as well as initiating apoptotic cell death that is associated or not associated with triggering the ROS accumulation (Vidya Priyadarsini et al., 2010; Alonso-Castro et al., 2013; Bishayee et al., 2013) (see Table 2). Induction of apoptosis is one of the strategies applied in the development of anticancer drugs to eliminate the malignant or infected cells. Two widely distributed flavonols, fisetin and quercetin are able also to suppress the migration and invasion of human cervical tumor cells providing some insights on the use of these compounds as potential antimetastatic agents in cancer chemotherapy (Bishayee et al., 2013; Chou et al., 2013).

Differently from some flavones described above, introduction of sugar moiety into the structure of flavonol kaempferol does not seem to result in any remarkable decrease in its cytotoxic activity as both kaempferol-7-O-β-D-glucoside and kaempferitrin display important time- and dose-dependent anticancer activity in human cervix carcinoma HeLa cells (Xu et al., 2008; Alonso-Castro et al., 2013).

Isoflavones

Isoflavones are non-nutritive polyphenolic compounds with genistein as the most abundant isoflavone found in soybeans and soy products (Dayuthapani et al., 2013). This isoflavone can reduce the viability of various human cervical cancer cell lines differing from one another in their HPV status and origin (see Table 2). Such anticancer action might work through numerous mechanisms, including arresting the cell cycle, initiating apoptotic changes and suppressing the invasive potential of tumor cells (Wang et al., 2001; Papazisis et al., 2006; Kim et al., 2009b; Zhou et al., 2009; Jha et al., 2010; Hussain et al., 2012; Dayuthapani et al., 2013).

Although some growth inhibitory activity of genistein is measured in various cervical cancer cell lines (HPV-free C33A, HPV16-positive CaSki and SiHa, HPV18-positive HeLa and KB-V1/Vbl, HPV68-positive ME180) the cytotoxic activity expressed by this compound is somewhat stronger in HeLa cells compared to CaSki and C33A cells (Kim et al., 2009b). Also, the sensitivity to this isoflavone is higher in HeLa cells compared to ME180 cells (Wang et al., 2001). The underlying factors rendering HeLa cells more sensitive to growth inhibition by genistein are interesting but still rather obscure. On the one hand, such results may reveal some selectivity of genistein to certain signaling components related to the infection of cells with HPV18 subtype. On the other hand, the stronger cytotoxic effect in adenocarcinoma cells compared to cervical squamous carcinoma cells is noteworthy and may have therapeutic implication to improve the treatment arsenal of adenomatous malignancies of uterine cervix.

Furthermore, somewhat stronger cytotoxic activity of genistein in CaSki cells compared to that of SiHa cells (Table 2) can be related to the differences in copies of HPV16 genome in the respective cell lines (CaSki cells contain up to 600 copies of integrated HPV16 per cell, whereas the number of copies in SiHa cells is only 1 or 2 per cell; Table 1).

Sensitization of Cervical Cancer Cells to Chemo- and/or Radiotherapy by Flavonoids

One of the main reasons for treatment failure of cervical carcinomas is emergence of resistance of tumor cells to conventional chemoradiation therapy leading to decreased therapeutic efficacy and indicating poor prognosis for patients (Jakubowicz-Gil et al., 2005; Zhang B et al., 2006; Kim et al., 2009b; Kim et al., 2009a; Lo et al., 2012; Lo et al., 2013; Singh et al., 2013). Also, the clinical use of current treatment modalities is often hampered by severe side effects and toxicity to normal tissues (Xu et al., 2008; Xu et al., 2011; Singh et al., 2013). Therefore, considering the high incidence and mortality rate of cervical cancer worldwide novel treatment strategies are urgently required (Zhang et al., 2006; Xu et al., 2008; Singh et al., 2013).

Numerous recent studies have shown that combining phytochemicals with standard cancer therapies may lead to improvement of overall effectiveness rendering malignant cells more sensitive to chemo- and radiotherapy and minimizing toxicity (Di Domenico et al., 2012; Lo et al., 2012; Singh et al., 2013). An increasing number of studies are focused on finding of natural compounds that can be combined with current drugs and several flavonoids are proved to sensitize cancer cells to death induced by antitumor agents (Ju et al., 2012).

The data about sensitization of human cervical cancer cells to conventional treatment modalities by natural flavonoids are summarized in Table 3. However, these data are still rather scarce, especially by comparing with the knowledge on some other malignancies, like for instance ovarian cancer.

Sensitization of cervical cancer cells to standard chemotherapeutics

Cisplatin is one of the most potent and widely used chemotherapeutic drugs for treatment of various solid tumors including cervical cancers; however, chemoresistance to this drug has remained a major limitation of cisplatin-based chemotherapy (Chung et al., 2010; He et al., 2012; Singh et al., 2013). Combination of

Table 3. Sensitization of Cervical Cancer Cells to Chemo- and Radiotherapy by Flavonoids

Flavonoid	Cell line	Drug/ therapy	Mechanism	Reference
EGCG	SiHa	Cisplatin	Enhancement in cytotoxicity, induction of apoptosis due to excessive ROS generation	Singh et al., 2013
	HeLa	Cisplatin	Enhancement in cytotoxicity, induction of apoptosis due to excessive ROS generation	Singh et al., 2013
Apigenin	HeLa	Paclitaxel	Synergistic cytotoxicity; sensitization of cells to paclitaxel-induced apoptosis through accumulation of ROS	Xu et al., 2011
Wogonin	HeLa	Cisplatin	Synergistic cytotoxicity; enhancement of apoptosis through triggering ROS accumulation	He et al., 2012
Quercetin	HeLa	Cisplatin	Sensitization of cells to cisplatin-induced apoptosis; the best temporal regime 0/24 quercetin/ cisplatin (in hours)	Jakubowicz-Gil et al., 2005
		Irradiation	Radiosensitizing enhancement ratio of 1.65	Lin et al., 2012
7, 3', 4'-trihydroxy-isoflavone (7, 3`, 4'-THIF)	HeLa	Epirubicin	Potentiation of cytotoxicity by increase in ROS levels and triggering apoptosis; enhancement of intracellular epirubicin accumulation	Lo et al., 2012
Formononetin	HeLa	Epirubicin	Potentiation of cytotoxicity by induction of ROS production and sensitization of cells to apoptosis; increase in intracellular epirubicin accumulation	Lo et al., 2013
Genistein	CaSki	Irradiation	Sensitization of cells to the death of irradiation; arresting cells in G2/M phase, stimulation of ROS production, induction of apoptosis; downregulation of E6 and E7 expression	Shin et al., 2008
		Irradiation	Radiation enhancement ratio from 1.7 to 3.9 at doses of 2.5-40.0μM genistein	Yashar et al., 2005
	HeLa	Irradiation	Enhancement of radiosensitivity by increasing the cells in G2/M phase and induction of apoptosis; the best temporal regime 0/48 genistein/ irradiation (in hours)	Zhang B et al., 2006
	ME180	Irradiation	Radiation enhancement ratio from 1.6 to 91.1 at doses of 2.5-40.0μM genistein; induction cell cycle arrest at G2/M phase	Yashar et al., 2005

cisplatin with other agents can be a promising strategy to overcome resistance and natural flavonoids may hold a great potential in such sensitization. In this way, green tea flavanol EGCG is able to chemosensitize human cervical cancer cells HeLa and SiHa to cisplatin-induced growth inhibition and apoptosis by excessive ROS generation (Singh et al., 2013). Also, wogonin can be used as a cisplatin sensitizer as cotreatment of cisplatin with this flavone results in synergistic cytotoxicity with significant enhancement of apoptotic death in HeLa cells (He et al., 2012). In addition, pretreatment with quercetin can make HeLa cells more vulnerable to apoptosis caused by cisplatin (Jakubowicz-Gil et al., 2005) supporting the potential use of these natural flavonoids as adjuvant of cisplatin.

Apigenin can sensitize HeLa cells to paclitaxel-induced apoptosis by enhancing the intracellular ROS accumulation. Such combined use might improve the efficiency of paclitaxel as a chemotherapeutic drug and could be an effective way to reduce the dosage of paclitaxel in cancer therapy accompanied by a decrease in its harmful side effects (Xu et al., 2011).

Two natural isoflavones, 7, 3`, 4`-trihydroxyisoflavone (7, 3`, 4`-THIF) and formononetin, are able to significantly increase the cytotoxicity of epirubicin in HeLa cells. Epirubicin is an anthracycline drug and its use in cancer treatment is often hampered by development of multidrug resistance. 7, 3`, 4`-THIF and formononetin can be used as adjuvants to enhance the chemosensitivity of epirubicin in cervical cancer cells and reverse the multidrug resistance, allowing thus to reduce the chemotherapy dosage and thereby also corresponding side effects (Lo et al., 2012; Lo et al., 2013).

Sensitization of cervical cancer cells to irradiation

Radiation is one of the critical treatment methods for cancer therapies and is widely used also for cervical carcinomas. The development of radioresistance is considered to be a major obstacle to the success of radiotherapy and overcoming this problem is still a big challenge for radiation oncologists (Yashar et al., 2005; Zhang B et al., 2006; Lin et al., 2012). Thus, there is an urgent need to develop radiosensitizers for enhancement of efficacy of radiotherapy.

A ubiquitous dietary flavonoid quercetin is shown to significantly increase the radiosensitivity in HeLa cells functioning as a powerful radiosensitizer (Lin et al., 2012). However, somewhat more data can be found about the radiosensitizing potential of common isoflavone genistein (Table 3). This compound can enhance the radiosensitivity of HeLa cells through increasing apoptosis and modulating cell cycle progression allowing to reduce the therapeutic doses of irradiation and minimize the adverse reactions (Zhang et al., 2006). Genistein behaves as a radiosensitizer also in another human cervical cancer cell line, CaSki. The cotreatment with genistein and irradiation leads to a remarkable decrease in cellular viability and induces apoptosis via ROS modulation; the commitment of

CaSki cells to undergo apoptosis might be attributed to the downregulation of E6 and E7 expression (Shin et al., 2008). However, the radiosensitizing potential of genistein might be variable in different human cervical cancer cell lines depending on the special cellular characteristics. In this way, HPV68-positive ME180 cells are shown to be more sensitive to genistein than HPV16-positive CaSki cells (Yashar et al., 2005). Although these cell lines represent two spectra of HPV infection, they both are derived from squamous carcinoma of uterine cervix (Table 1). Therefore, it is possible that sensitization of cervical cancer cells to chemoradiation can be determined by HPV subtype related factors and these mechanisms certainly need further molecular studies. By all means, it is evident that genistein-modulated cytotoxicity may provide a novel basis for radiation therapy and has the potential to benefit cervical cancer patients.

Summary and Further Perspectives

Numerous natural flavonoids from different structural classes express cytotoxic activity in various human cervical cancer cell lines providing thus new perspectives in drug development against this devastating disease. Such antitumor action involves multiple molecular mechanisms, including modulation of cell cycle progression, promotion of apoptotic cell death, suppression of migration and invasion of malignant cells, and interfering with angiogenetic processes.

The anticarcinogenic activity of flavonoids may differentially depend on the cellular type and origin of cervical carcinoma. Indeed, green tea flavanol EGCG seems to be somewhat more active in squamous cell carcinoma cell lines compared to adenocarcinoma lines, whereas widely distributed flavone luteolin and isoflavone genistein reveal higher activity on adenocarcinoma cell line, HeLa compared to its squamous cell counterparts.

Several natural flavonoids are able to inhibit the interactions between oncoproteins (E6 and E7) and tumor suppressors (p53 and pRb) and thereby decrease the growth of immortalized cell lines containing different HPV types. Some flavones can even reveal specificity toward certain HPV strains as jaceosidin is proved to specifically inhibit HPV16-harboring cell lines showing no growth inhibition in HPV18-positive and HPV-negative cells, and luteolin can exert some specificity to HPV18-containing cells being only weakly active in HPV16-harboring and HPV-free cells. If further confirmed, such differential activity might be used in targeting and treatment of preinvasive and invasive lesions of uterine cervix associated with infection of certain HPV subtypes. Moreover, these data also raise a question about the potential antitumor effects of flavonoids in human cervical cancer cells harboring other oncogenic HPV types. Therefore, further experiments with immortalized cell lines containing various different high-risk HPV strains can be interesting and are certainly required, especially considering the situation where prophylactic vaccines are restricted to applications only for two oncogenic HPV types (16 and 18). The data about differential cytotoxic activity of isoflavone genistein in HPV18-positive HeLa cells and HPV68-positive ME180 cells allow to suppose that such strain-specific anticancer effects of flavonoids might indeed appear, and to efficiently intervene to cervical carcinogenesis it might be important to consider these effects.

One more factor that can be involved in determining the cytotoxic potency of flavonoids in cervical cancer cells is the copy number of integrated HPV genome per cell. In this way, several flavonoids (isoliquiritigenin, amentoflavone, genistein) tend to display somewhat higher cytotoxicity in CaSki cells (harboring up to 600 copies of HPV16 DNA per cell) compared to SiHa cells which contain only 1-2 copies of HPV16 per cell.

It is evident that current knowledge about the molecular cytotoxic mechanisms of flavonoids in human cervical cancer cells is still rather scarce. Nevertheless, it is also clear that flavonoids may have future utility in clinical applications for treating cervical cancer.

Besides the cytotoxic activity per se, several flavonoids are able also to sensitize cervical cancer cells to conventional chemoradiation therapy, hampering the emergence of resistance of tumor cells to current treatment modalities. Such combined approach could improve the efficiency of standard therapies and allow to reduce the doses of chemotherapy drugs and irradiation leading to decrease in corresponding adverse side effects. Although few, current data about the chemo- and radiosensitizing action of flavonoids still clearly suggest the potential use of natural polyphenols as adjuvants of chemotherapeutics (cisplatin, paclitaxel, epirubicin) as well as radiotherapy. Moreover, like cytotoxic activity also the sensitizing potential of flavonoids might be variable in human cervical cancer cells containing different HPV subtypes. The differential radiosensitizing potential of isoflavone genistein in HPV16-positive CaSki cells and HPV68-positive ME180 cells provides some support to this conception; however, it is clear that further experimental data are certainly needed.

Considering the high incidence and mortality rate of cervical cancer but also the side effects associated with conventional treatments and acquired resistance to standard therapies, the need for novel treatment modalities is tremendous. Polyphenolic compounds are broadly distributed in the plant kingdom and it is possible that several hitherto uncharacterized flavonoids with potential anticancer activity and/or chemo- or radiosensitizing properties are still waiting for their identification. Therefore, it is crucial to keep up these studies for searching new promising leads for novel therapeutic drugs from natural/herbal resource as well as investigating their action mode in cervical tumor cells with different molecular characteristics.

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