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

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...
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 onogenic 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 onogenic 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; Jin et al., 2012; Alshatwi et al., 2013; Ramesh and Alshatwi, 2011; Lee et al., 2011; Di Domenico et al., 2012; Meiyanto et al., 2012; Alonso-Castro et al., 2013). 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 (Cheng 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., 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 Priyadarshini 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; Kna, 2013; Kundu and Chun, 2014). These 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 (Cheng 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., 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.
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Characteristics of Cytotoxicity of Flavonoids on Cervical Cancer Cells

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 carcinomas 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 chemotherapy 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, flavonols, flavones, flavonoids, 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

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Table 1. Characteristics of Human Cervical Cancer Cell Lines

| Cell line | HPV status | Further description                                      | References |
|-----------|------------|----------------------------------------------------------|------------|
| C33A      | Negative   | p53 mutated                                              | Lee et al., 2005; Hirchauld 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; Hirchauld 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; Gao et al., 2004; Totta et al., 2004; Virgili et al., 2004; Zhang B et al., 2006; Hirchauld 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 |

The potency of anticancer action of EGCG in human cervical cancer 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).
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 3.91           | MTT 72            | Induction of G2/M phase cell cycle arrest and apoptosis | Hirchund et al., 2013 |
| SiHa 40             | SBB 24            | Induction of apoptosis by increase in ROS levels | Yuan X et al., 2012 |
| HeLa 58.1-82.6 (70.4) | MTT 72         | Induction of G2/M phase cell cycle arrest and apoptosis | Li et al., 2008; Hirchund et al., 2013 |
| **Flavonol**        |                   |               |              |                 |            |
| EGCG OMC-4 4.1      | Cell counts 48    | Induction of apoptosis | Noguchi et al., 2006 |
| CaSi 137 (>100)     | MTT 72            | Induction of apoptosis | Liu et al., 2011; Liu et al., 2011 |
| SiHa 223-243 (233)  | MTT 48            | Induction of apoptosis | Kim et al., 2010; Kim et al., 2012 |
| **Flavanone**       |                   |               |              |                 |            |
| Hesperetin SiHa     | 650               | MTT 24        | Induction of G2/M phase cell cycle arrest and apoptosis | Ashawati et al., 2013 |
| **Flavonol**        |                   |               |              |                 |            |
| Luteolin CaSi       | >94               | MTT 72        | Induction of apoptosis | Wang et al., 2011 |
| SiHa 40             | SBB 24            | Induction of apoptosis | Yuan X et al., 2012 |
| HeLa 58.1-82.6 (70.4) | MTT 72         | Induction of G2/M phase cell cycle arrest and apoptosis | Li et al., 2008; Hirchund et al., 2013 |

Note: *MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SBB = MTS = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; WST-1 = 2-(2-methoxyphenyl)-3-(4-sulfophenyl)-5-(4-sulfophenyl)-2H-tetrazolium, inner salt; SRB = sulforhodamine B; TMCC-1 = 2-(2-methoxyphenyl)-3-(4-sulfophenyl)-5-(4-sulfophenyl)-2H-tetrazolium, inner salt; OMC-4 = 2-(2-methoxyphenyl)-3-(4-sulfophenyl)-5-(4-sulfophenyl)-2H-tetrazolium, inner salt.

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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., 2011; Kim MS et al., 2013). These data suggest that 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 of HPV-non-harboring C33A cells (Lee et al., 2011). Some differences in the sensitivity of this biflavone to CaSkii and SiHa cells are probably associated with variations in the copy numbers of integrated HPV16 genome, as CaSkii 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, CaSkii) 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 CaSkii 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

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 cytotoxicity 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 CaSkii 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 CaSkii and SiHa cells are probably associated with variations in the copy numbers of integrated HPV16 genome, as CaSkii 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, CaSkii) 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.

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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 Priyadarshini 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 antitemetastic agents in cancer chemotheraphy (Bishayee et al., 2013; Chou et al., 2013).

Differently from some flavonones 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 kaempferitin 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
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

### 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'-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 |
|           | HeLa      | 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 |
Characteristics of Cytotoxicity of Flavonoids on Cervical Cancer Cells

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 angiogenic 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 chemotherapeutics 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 chemotherapeutics and 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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References

Ahn WS, Huh SW, Bae SM, et al (2003a). A major constituent of green tea, EGCG, inhibits the growth of a human cervical
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Cancer cell line, CaSkii cells, through apoptosis, G(1) arrest, and regulation of gene expression. DNA Cell Biol, 22, 217-24.

Ahn WS, Yoo J, Huh SW, et al (2003b). Protective effects of green tea extracts (polyphenol E and EGCG) on human cervical lesions. Eur J Cancer Prev, 12, 383-90.

Al-Hazzani AA, Alshawi AA (2011). Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells. Food Chem Toxicol, 49, 3281-6.

Alonso-Castro AJ, Ortiz-Sanchez E, Garcia-Regalado A, et al (2013). Kaempferitin induces apoptosis via intrinsic pathway in HeLa cells and exerts antitumor effects. J Ethnopharmacol, 145, 476-89.

Alshawi AA, Ramesh E, Periasamy VS, Subash-Babu P (2013). The apoptotic effect of hesperitin on human cervical cancer cells is mediated through cell cycle arrest, death receptor, and mitochondrial pathways. Fundam Clin Pharmacol, 27, 581-92.

Bazzaro M, Ancic Iva P, Mustajic MK, et al (2011). α,β-Unsaturated carboxyl system of chalcone-based derivatives is responsible for broad inhibition of proteasomal activity and preferential killing of human papilloma virus (HPV) positive cervical cancer cells. J Med Chem, 54, 449-56.

Bhatia N, Zhao J, Wolf DM, Agarwal R (1999). Inhibition of human cervical cancer cells mediated by green and black tea in relation to chemoprevention and gynecologic cancers. Mol Nutr Food Res, 55, 931-40.

Cardenas M, Marder M, Blanck VC, Roguin LP (2006). Antitumor activity of some natural flavonoids and synthetic derivatives on various human and murine cancer cell lines. Bioorg Med Chem, 14, 2966-71.

Chen D, Cao J, Tian L, Liu F, Sheng X (2011). Induction of apoptosis by EGCG in selected tumour cell lines in vitro. Folia Histochem Cytobiol, 49, 229-32.

Butler LM, Wu AH (2011). Green and black tea in relation to cervical adenocarcinoma HeLa cells. J Food Funct, 8, 1224-7.

Cherry JJ, Rietz A, Malinkevich A, et al (2013). Structure based identification and characterization of flavonoids that disrupt the apoptotic pathway. Basic Clin Pharmacol Toxicol, 113, 46-53.

Chung KS, Choi JH, Back NI, et al (2010). Eupafolin, a flavonoid from Morus alba. Fitoterapia, 81, 1224-7.

Czyz J, Madeja Z, Irmer U, Korohoda W, Hülsner DF (2005). Flavonoid apigenin inhibits motility and invasiveness of carcinoma cells in vitro. Int J Cancer, 114, 12-8.

Dat NT, Binh PT, Quynh LT, et al (2010). Cytotoxic prenylated flavonoid from Morus alba. Fitoterapia, 81, 1224-7.

Deng X, Zhao X, Lan Z, et al (2014). Anti-tumor effects of flavonoids from the ethnic medicine Docienia delavayi (Franch.) Schnid. and its possible mechanism. J Med Food, 17, 787-94.

Dhandayuthapani S, Marimuthu P, Hörmann V, Kami-Diaka J, Rathinavelu A (2013). Induction of apoptosis in HeLa cells via caspase activation by resveratrol and genistein. J Med Food, 16, 139-46.

Di Domenico F, Foppoli C, Coccia R, Perluigi M (2012). Antioxidants in cervical cancer: chemopreventive and chemotherapeutic effects of polyphenols. Biochim Biophys Acta, 1822, 737-47.

Garcia FA, Cornelison T, Nuno T, et al (2014). Results of a phase II randomized, double-blind, placebo-controlled trial of polyphenon E in women with persistent high-risk HPV infection and low-grade cervical intraepithelial neoplasia. Gynecol Oncol, 132, 377-82.

GLOBOCAN2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Default.aspx

Guo JM, Kang GZ, Xiao BX, Liu DH, Zhang S (2004). Effect of daidzein on cell growth, cell cycle, and telomerase activity of human cervical cancer in vitro. Int J Gynecol Cancer, 14, 882-8.

Ham S, Kim KH, Kwon TH, et al (2014). Luteolin induces intrinsic apoptosis via inhibition of E6/E7 oncoproteins and activation of extrinsic and intrinsic signaling pathways in HPV-18-associated cells. Oncol Rep, 31, 2683-91.

He F, Wang Q, Zheng XL, et al (2012). Wogonin potentiates cisplatin-induced cervical cancer cell apoptosis through accumulation of intracellular reactive oxygen species. Oncol Rep, 28, 601-5.

Hernandez BY, McDuffie K, Franke AA, Killeen J, Goodman MT (2004). Reports: plasma and dietary phytoestrogens and risk of premalignant lesions of the cervix. Nutr Cancer, 49, 109-24.

Hirchaud F, Hermetet F, Ablise M, et al (2013). Isoliquiritigenin induces caspase-dependent apoptosis via downregulation of HPV E6 E6 expression in cervical cancer CaSkii cells. Planta Med, 79, 1628-35.

Hsu YL, Chia CC, Chen PJ, et al (2009). Shallot and lycorice constituent isoliquiritigenin arrests cell cycle progression and induces apoptosis through the induction of ATM/p53 and initiation of the mitochondrial system in human cervical carcinoma HeLa cells. Mol Nutr Food Res, 53, 826-35.

Hu T, He XW, Jiang JG, Xu XL (2014). Efficacy evaluation of a Chinese bitter tea (Ilex latifolia Thunb.) via analyses of its main components. Food Funct, 5, 876-81.

Hussain A, Harish G, Prabhu SA, et al (2012). Inhibitory effect of genistein on the invasive potential of human cervical cancer cells via modulation of matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 expression. Cancer Epidemiol, 36, 387-93.

Jakurowicz-Gil J, Paduch R, Piersiak T, et al (2005). The effect of querctin of pro-apoptotic activity of cisplatin in HeLa cells. Biochem Pharmacol, 69, 1343-50.

Jha AK, Nikbakht M, Parashar G, et al (2010). Reversal of hypermethylation and reactivation of the RARβ2 gene by natural compounds in cervical cancer cell lines. Folia Biol (Praga), 56, 195-200.

Jin YM, Xu TM, Zhao YH, Wang YC, Cui MH (2014). In vitro and...
in vivo anti-cancer activity of formononetin on human cervical cancer cell line HeLa. *Tumour Biol.*, **35**, 2279-84.

Ju HK, Lee HW, Chung KS, et al (2012). Standardized flavonoid-rich fraction of Artemisia princeps Pampampanin cv. Sajabal induces apoptosis via mitochondrial pathway in human cervical cancer HeLa cells. *J Ethnopharmacol.*, **141**, 460-8.

Jung JH, Lee JO, Kim JH, et al (2010). Quercetin suppresses HeLa cell viability via AMPK-induced HSP70 and EGFR down-regulation. *J Cell Physiol.*, **223**, 408-14.

Kanno S, Tomizawa A, Hiura T, et al (2005). Inhibitory effects of liquiritigenin on tumor growth in human cancer cell lines and sarcoma S-180-implanted mice. *Bioll Pharm Bull.*, **28**, 527-30.

Kim EY, Kim AK (2013). Combination effect of equal and TRAIL against human cervical cancer cells. *Anticancer Res.*, **33**, 903-12.

Kim H, Moon JY, Mosaddik A, Cho SK (2010). Induction of apoptosis in human cervical carcinoma HeLa cells by polymethoxylated flavone-rich Citrus grandis Osbeck (Danggyuja) leaf extract. *Food Chem Toxicol.*, **48**, 2435-42.

Kim JH, Kang JW, Kim MS, et al (2012). The apoptotic effects of the flavonoid N101-2 in human cervical cancer cells. *Toxicol In Vitro*, **26**, 67-73.

Kim MS, Baik Y, Park YS, et al (2013). Wogonin induces apoptosis by suppressing E6 and E7 expressions and activating intrinsic signaling pathways in HPV-16 cervical cancer cells. *Cell Biol Toxicol.*, **29**, 259-72.

Kim SH, Kim SH, Kim YB, et al (2009a). Genistein inhibits cell growth by modulating various mitogen-activated protein kinases and AKT in cervical cancer cells. *Ann N Y Acad Sci.*, **1171**, 495-500.

Kim SH, Kim SH, Lee SC, Song YS (2009b). Involvement of both extrinsic and intrinsic apoptotic pathways in apoptosis induced by genistein in human cervical cancer cells. *Ann N Y Acad Sci.*, **1171**, 196-201.

Kitdamrongtham W, Manosroi A, Akazawa H, et al (2013). Potent anti-cervical cancer activity: synergistic effects of Thai medicinal plants in recipe N040 selected from the MANOSROI III database. *J Ethnopharmacol.*, **149**, 288-96.

Kma L (2013). Roles of plant extracts and constituents in cervical cancer therapy. *Asian Pac J Cancer Prev.*, **14**, 3429-36.

Koppikar SJ, Choudhari AS, Suryavanshi SA, et al (2010). Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. *BMC Cancer*, **10**, 210.

Krifa M, Alhosin M, Muller CD, et al (2013). Limoniastrium guynonianum aqueous gall extract induces apoptosis in human cervical cancer cells involving p16 INK4A re-expression related to UHRF1 and DNM1 down-regulation. *J Exp Clin Cancer Res.*, **32**, 30.

Kundu JK, Chun KS (2014). The promise of dried fruits in cancer chemoprevention. *Asian Pac J Cancer Prev.*, **15**, 3343-52.

Kuo YJ, Hwang SY, Wu MD, et al (2008). Cytotoxic constituents from Podocarpus fasciculus. *Chem Pharm Bull (Tokyo)*, **56**, 585-8.

Lee HG, Yu KA, Oh WK, et al (2005). Inhibitory effect of jaceosidin isolated from Artemisiaargyi on the function of E6 and E7 oncoproteins of HPV16. *J Ethnopharmacol.*, **98**, 339-43.

Lee S, Kim H, Kang JW, et al (2011). The biflavonoid amentoflavone induces apoptosis via suppressing E7 expression, cell cycle arrest at sub-G1 phase, and mitochondria-emanated intrinsic pathways in human cervical cancer cells. *J Med Food.*, **14**, 808-16.

Li F, Awale S, Tezuka Y, Kadota S (2008). Cytotoxic constituents from Brazilian red propolis and their structure-activity relationship. *Bioorg Med Chem.*, **16**, 5434-40.

Li F, Awale S, Tezuka Y, Kadota S (2010). Cytotoxicity of constituents from Mexican propolis against a panel of six different cancer cell lines. *Nat Prod Commun.*, **5**, 1601-6.

Li HN, Nie FF, Liu W, et al (2009). Apoptosis induction of oxoynil A in human cervical cancer HeLa cell line in vitro and in vivo. *Toxicology*, **257**, 80-5.

Lin C, Yu Y, Zhao HG, et al (2012). Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiother Oncol.*, **104**, 395-400.

Liu C, Wang Y, Xie S, et al (2011). Liquiritigenin induces mitochondria-mediated apoptosis via cytochrome c release and caspases activation in HeLa cells. *Phytother Res.*, **25**, 277-83.

Liu Y, Xie S, Wang Y, et al (2012). Liquiritigenin inhibits tumor growth and vascularization in a mouse model of HeLa cells. *Molecules.*, **17**, 7206-16.

Lo YL, Wang W (2013). Formononetin potentiates epirubicin-induced apoptosis via ROS production in HeLa cells in vitro. *Chem Biol Interact.*, **205**, 188-97.

Lo YL, Wang W, Ho CT (2012). 7, 3′, 4′-Trihydroxyisoflavone modulates multidrug resistance transporters and induces apoptosis via production of reactive oxygen species. *Toxicology.*, **302**, 221-32.

Mamadalieva NZ, Herrmann F, El-Readi MZ, et al (2011). Flavonoids in Scutellaria immuculata and S. ramosissima (Lamiaceae) and their biological activity. *J Pharm Pharmacol.*, **63**, 1346-57.

Meiyanto E, Hermawan A, Anindiyaji (2012). Natural products for cancer-targeted therapy: citrus flavonoids as potent chemopreventive agents. *Asian Pac J Cancer Prev.*, **13**, 427-36.

Moga MA, Irimie M, Oanta A, Pascu A, Burtea V (2014). Type-specific prevalence of human papillomavirus by cervical cytology among women in Brasov, Romania. *Asian Pac J Cancer Prev.*, **15**, 6887-92.

Muthusami S, Prabakaran DS, An Z, Yu JR, Park WY (2013). EGCG suppresses Fused Toes Homolog protein through p53 in cervical cancer cells. *Mol Biol Rep.*, **40**, 5587-96.

Noguchi M, Yokoyama M, Watanabe S, et al (2006). Inhibitory effect of the tea polyphenol, (-)-epigallocatechin gallate, on growth of cervical adenocarcinoma cell lines. *Cancer Lett.*, **234**, 135-42.

Noh HJ, Sung EG, Kim JY, Lee TJ, Song IH (2010). Suppression of phorbol-12-myristate-13-acetate-induced tumor cell invasion by apigenin via the inhibition of p38 mitogen-activated protein kinase-dependent matrix metalloproteinase-9 expression. *Oncol Rep.*, **24**, 277-83.

O’Rey J, Brown J, Fleming J, Harrison PR (2003). Effects of dietary flavonoids on major signal transduction pathways in human epithelial cells. *Biochem Pharmacol.*, **66**, 2075-88.

Papazisis KT, Kalemic TG, Goldsborough AS, Callaghan R, Moga MA, Irimie M, Oanta A, Pascu A, Burtea V (2014). Synergistic effects of protein tyrosine kinase inhibitor genistein with dietary flavonoids on major signal transduction pathways in human cervical cancer cells. *Cancer Lett.*, **327**, 277, 174-81.

Pluchino KM, Hall MD, Goldsborough AS, Callaghon R, Gottesman MM (2012). Collateral sensitivity as a strategy against cancer multidrug resistance. *Drug Resist Updat.*, **15**, 98-105.

Qiao Y, Cao J, Xie L, Shi X (2009). Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells. *Arch Pharm Res.*, **32**, 1309-15.

Ramesh E, Alshawi AA (2013). Naringin induces death receptor and mitochondria-mediated apoptosis in human cervical cancer (SiHa) cells. *Food Chem Toxicol.*, **51**, 97-105.

Sah JF, Balasubramanian S, Eckert RL, Rorkke EA (2004). Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. *J Biol Chem.*, **279**, 12755-62.

Samama B, Plas-Roser S, Schaeffer C, et al (2002). HPV DNA detection by in situ hybridization with catalyzed signal amplification on thin-layer cervical smears. *J Histochem Cytochem.*, **50**, 1417-20.
Yang L, Zheng XL, Sun H, et al (2011). Catalase suppression-mediated H2O2 accumulation in cancer cells by wogonin effectively blocks tumor necrosis factor-induced NF-κB activation and sensitizes apoptosis. *Cancer Sci*, 102, 870-6.

Yang LL, Chang CC, Chen LG, Wang CC (2003). Antitumor principle constituents of Myrica rubra Var. acuminata. *J Agric Food Chem*, 51, 2974-9.

Yashar CM, Spanos WJ, Taylor DD, Gercel-Taylor C (2005). Potentiation of the radiation effect with genistein in cervical cancer cells. *Gynecol Oncol*, 99, 199-205.

Ying TH, Yang SF, Tsai SJ, et al (2012). Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8/caspase-3-dependent pathway. *Arch Toxicol*, 86, 263-73.

Yokoyama M, Noguchi M, Nakao Y, et al (2008). Antiproliferative effects of the major tea polyphenol, (-)-epigallocatechin gallate and retinoic acid in cervical adenocarcinoma. *Gynecol Oncol*, 108, 326-31.

Yokoyama M, Noguchi M, Nakao Y, Pater A, Iwasaka T (2004). The tea polyphenol, (-)-epigallocatechin gallate effects on growth, apoptosis, and telomerase activity in cervical cell lines. *Gynecol Oncol*, 92, 197-204.

Yuan CH, Filippova M, Tungteakkhun SS, Duerksen-Hughes PJ, Krstenansky JL (2012). Small molecule inhibitors of the HPV16-E6 interaction with caspase 8. *Bioorg Med Chem Lett*, 22, 2125-9.

Yuan X, Zhang B, Chen N, et al (2012). Issiliqutigignen treatment induces apoptosis by increasing intracellular ROS levels in HeLa cells. *J Asian Nat Prod Res*, 14, 789-98.

Yuan X, Zhang B, Gan L, et al (2013). Involvement of the mitochondrion-dependent and the endoplasmic reticulum stress-signaling pathways in isoliquriquiginen-induced apoptosis of HeLa cell. *Biomed Environ Sci*, 26, 268-76.

Zeng F, Tian L, Liu F, et al (2012a). Induction of apoptosis by casticin in cervical cancer cells: reactive oxygen species-dependent sustained activation of Jun N-terminal kinase. *Acta Biochem Biophys Sin (Shanghai)*, 44, 442-9.

Zeng XT, Xiong PA, Wang F, et al (2012b). Passive smoking and cervical cancer risk: a meta-analysis based on 3230 cases and 2982 controls. *Asian Pac J Cancer Prev*, 13, 3687-93.

Zhang B, Liu JY, PAM JS, et al (2006). Combined treatment of ionizing radiation with genistein on cervical cancer HeLa cells. *J Pharmacol Sci*, 102, 129-35.

Zhang Q, Tang X, Lu Q, et al (2006). Green tea extract and (-)-epigallocatechin-3-gallate inhibit hypoxia- and serum-induced HIF-1α protein accumulation and VEGF expression in human cervical carcinoma and hematoma cells. *PLoS One*, 7, 10255.

Zhang T, Chen X, Qu L, et al (2004). Chrysin and its phosphate ester inhibit cell proliferation and induce apoptosis in HeLa cells. *Bioorg Med Chem*, 12, 6907-15.

Zhang T, Du J, Liu L, et al (2012). Inhibitory effects and underlying mechanism of 7-hydroxyflavone phosphate ester in HeLa cells. *PLoS One*, 7, 006522.

Zhang X, Xu Y, Chen Y, et al (2012). Cellular and molecular mechanisms of silibinin induces cell-cycle arrest and apoptosis on HeLa cells. *Cell Biochem Funct*, 30, 243-8.

Zhang PW, Chiang LC, Lin CC (2005). Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. *Life Sci*, 76, 1367-79.

Zhou N, Yan Y, Li W, et al (2009). Genistein inhibition of topoisomerase Alphal expression participated by Sp1 and Sp3 in HeLa cell. *Int J Mol Sci*, 10, 3255-68.

Zhu X, Wang J, Ou Y, Han W, Li H (2013). Polyphenol extract of Phyllanthus emblica (PEEP) induces inhibition of cell proliferation and triggers apoptosis in cervical cancer cells. *Eur J Med Res*, 18, 46.

Zou C, Liu H, Feugang JM, et al (2010). Green tea compound in chemoprevention of cervical cancer. *Int J Gynecol Cancer*, 20, 617-24.