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Phosphatidylinositol 3-kinase Signaling as a Therapeutic Target for Cervical Cancer

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Abstract: Cervical cancer is the second most frequent cause of female cancer mortality and remains a major health problem in women worldwide. Surgery, chemotherapy and radiotherapy alone or combined are the three treatments methods commonly used to treat this disease. However, a significant proportion of the cancer patients still experiences recurrence and eventually dies. Recently, the research advances in molecular profiling and genomics have revealed that the phosphatidylinositol 3-kinases (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway plays a crucial role in mediating multiple cellular functions including cell growth, proliferation, metabolism, survival and angiogenesis. Thus, targeting this signal pathway offers a promising perspective for cervical cancer therapy. In this article, we review the published data from both basic and clinical studies showing that the concurrent cervical cancer chemoradiotherapy dramatically improves the local control of this disease and overall survival by triggering tumor cell apoptotic pathways *via* the PI3K/AKT/mTOR signalings, proving that the PI3K/AKT/mTOR pathway is one of the most important targets for cervical cancer therapy. We also highlight that several phytochemicals strongly inhibit proliferation of the cervical cancer cells and induce apoptosis by targeting one or multiple molecules through the PI3K/AKT/mTOR pathway. While some of these phytochemicals have been used as therapeutic agents or chemoradiotherapy sensitizers, others are currently in clinical development to be the potential therapeutic agents for the advanced cervical cancer therapy.

Keywords: Cervical cancer, Chemoradiotherapy, PI3K/AKT/mTOR signalings, Growth factors, Notch signalings, PI3K inhibitors, Phytochemicals, Chemoradiosensitizers.

INTRODUCTION

Cancer that forms in tissues of the cervix (the organ connecting the uterus and vagina) is called cervical cancer. Cervical cancer is the second most frequent cause of female cancer mortality worldwide with 288,000 deaths yearly. About 510,000 cases of cervical cancer are reported each year with nearly 80% in developing countries: 68,000 in Africa, 77,000 in Latin America, and 245,000 in Asia (http://www.who.int). Cervical cancer is caused by infection of high risk human papillomavirus (HPVs) [1, 2]. Two high risk types of HPVs, HPV16 and HPV18 are the causative agent for virtually over 95% of cervical cancer cases [1]. HPVs are non-enveloped, epitheliotropic, circular double-stranded DNA viruses, which contain five ORFs (E1, E2, E5, E6 and E7) encoding proteins expressed from promoters designated as "early" genes. Both E6 and E7 genes have been demonstrated to be the most important pathogenic genes called as oncogenes. The persistent expression of HPV16 E6 and E7 oncoproteins has been shown to be necessary to transform primary human keratinocytes *in vitro* [3]. Expression of the viral oncoproteins in high-risk HPV-infected cells results in chromosomal instability and accumulation of mutational events. These "endogenous" modifications are very important in the pathogenesis of premalignant lesions and tumour progression [1]. Recently, the development and availability of "cancer vaccines" against HPVs hold enormous promise for preventing HPV-associated cervical cancer, particularly in regions of the world where cytology based screening programs are inaccessible [4]. However, cervical cancer still remains a major health problem in women worldwide because of no therapeutic HPV vaccines. Surgery combined with radiation or chemotherapy is the major mean used for curative therapy of the cervical cancer.

Recent advances in molecular profiling have revealed that chromosomal instability and accumulation of mutational events drive the development of cervical cancer and other tumours through the phosphatidylinositol 3-kinases (PI3K)/ AKT/mammalian target of rapamycin (mTOR) pathway. The PI3K/AKT/mTOR pathway mediates multiple cellular functions critical to tumor initiation, progression and outcomes, including growth and proliferation, metabolism, motility, migration, invasion, angiogenesis, survival, and autophagy [5]. In this paper, we first briefly review how the PI3K/AKT/mTOR signaling pathway is involved in the cervical carcinogenesis, then discuss the basic and clinical

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evidence that the PI3K/AKT/mTOR signalings are the important therapeutical targets for cervical cancer therapy and finally highlight that phytochemicals targeting the PI3K/AKT/mTOR pathway are currently being evaluated and developed as potential cervical cancer therapeutics.

PI3K SIGNALING-MEDIATED CERVICAL CARCINOGENESIS

PIK3CA Modulation

PIK3CA is known as a regulator of PI3K, which plays an important role in the PI3K/AKT/mTOR signaling pathway contributing to tumorigenesis [6]. Previous studies have provided evidence that PIK3CA functions as an oncogene in cervical cancer [7]. The putative oncogene PIK3CA located at chromosome 3q24-29 encodes a PI3K commonly observed in cervical neoplasia. PIK3CA encodes the p110α catalytic subunit of PI3K to modulate different signalings important for cell proliferation, apoptosis and progression of neoplasia. This PI3K catalytic subunit is amplified in cervical cancers to play a key role in cervical carcinogenesis [6]. Somatic mutations in this gene have been detected in several solid human tumors including cervical cancer [8]. Oncogenic mutations of PIK3CA activate the PI3K/AKT/mTOR pathway in various malignancies. PCR-based DNA sequencing analysis reveals that PIK3CA mutations are the most frequent in squamous cervical cancer, 5 out of 14 cervical patients showed PIK3CA mutations (36%) [9]. Comparative genomic hybridization reveals further that amplification of the chromosome arm 3q is the most consistent chromosomal aberration, and is implicated in the progression of dysplastic uterine cervical cells into invasive cancer in primary tissues of cervical carcinoma [7]. An increased copy number of PI3KCA is positively correlated with 3q26.3 amplification in both cervical tumor tissues and cancer cell lines. Quantitative RT-PCR analysis shows that the PIK3CA gene copy number is 3 or more in 28 out of 40 cases [10]. Furthermore, 39 out of the 46 examined cervical neoplastic tissues show that $AKT^{(ser473)}$ is phosphorylated, revealing that PI3K generates inositol phospholipids to trigger AKT phosphorylation, which in turn affects tumor-driving signals [10].

Growth Factor Mediated-PI3K Pathway

Several growth factors as upstream regulators of the PI3K pathway have been implicated in promoting mitogenic, metastatic and antiapoptotic phenotypes in different types of cancers [11, 12]. Experimental evidence has revealed that expression of EGFR components correlates with clinical behavior of early-stage cervical cancer. According to the analysis of tumour samples from 336 cervical cancer patients all treated primarily by radical surgery, 32.1% patients express EGFR, 21.0% pEGFR and 38.3% PTEN [11]. In early-stage cervical cancer, loss of PTEN expression is associated with pelvic lymph node metastasis because PTEN is one of the tumor suppressor genes that can prevent pelvic lymph node metastasis [11]. It has been reported that tumour expression of lymphangiogenic growth factors is implicated in human cervical cancer progression [12]. IGF-1 system may have the adverse impacts on prognosis by IGF-1 receptor overexpression implicated in early-stage cervical cancer [13]. HPV16 E6- and E7-transfected cervical cancer cells express high levels of both HIF-1α and VEGF proteins, which can be abrogated by cotransfection with either HIF-1α siRNA or resveratrol treatment. Blockage of ERK 1/2 and PI3K by drugs PD98059 and LY294002 can also abolish 16 E6- and E7-induced expressions of both HIF-1α and VEGF, respectively [14]. An *in vitro* experiment has shown that IGF-1 stimulates growth and invasiveness of cervical cancer cells (SiHa and CaSki), but not those of normal cervical epithelial cells [15]. IGF-1 receptor protein is abundant in cervical cancer cell lines, in contrast, this protein is scarcely detected in normal cervical epithelial cells [15]. IGF-1 treatment triggers PI3K and MAPK cascades leading to the activation of AKT and Erk1/2 in cervical cancer cells. Erk1/2 siRNA transfection reduces its protein expression to abolish IGF-1-stimulated activity of KC1 co-transport-1 (KCC1) [16]. IGF-II can dramatically enhance mRNA and protein expression of KCC1 in SiHa cells, to activate protein phosphorylation in the PI3K/AKT and ERK1/2MAPK signal transduction pathways [17]. In addition, cervical cancer cell lines show different expression patterns of IGF-1 receptor and an isoform of the insulin receptor, IR-A[18]. While HPV-positive SiHa cells express IGF-IR, IR-A and IR-B, and IR/IGF-IR hybrid receptors, HPV-negative C33a cells only express the IR-A although the PI3K/MAPK pathways are active in both SiHa and C33a cells [18].

Normal cervical epithelium is negative for CXCR4 expression, in contrast, the human cervical carcinoma tissues (HCC) of cervical cancer patients highly express CXCR4 on their surface and on tumor cells [19]. Based on the analysis of CXCR4 expression in 85 archived tissues from clinical stage IB cervical adenocarcinoma (n = 37) and from benign specimens (n = 48), all cervical adenocarcinoma tissues and 75% of benign specimens show strong CXCR4 staining, only 25% of the benign specimens have weak or negative staining for CXCR4 [20]. CXCR4 expression is associated with cervical adenocarcinoma cell migration and proliferation, and primary cervical adenocarcinoma cells expressing CXCR4 are significantly more likely to metastasize to pelvic lymph nodes [20]. The metastasis is apparently mediated by the activation and phosphorylation of the AKT/ERK½ pathways in which SDF-1-CXCR4 axis plays a pivotal role [20]. It has been reported that SDF-1 chemoattracts HCC cells and enhances their scattering. SDF-1 also stimulates nuclear localization of β-catenins, upregulates its target gene cyclin D1 and activates RAS-MAPK, PI3K/AKT and JAK-STAT pathways [19]. The significant signal transduction events provoked by SDF-1 include chemotaxis and rescue from apoptosis. It has been noted that the SDF-1-mediated functions are additionally enhanced in the presence of HGF [19]. In addition, there are three lysophosphatidic acid (LPA)-specific receptors LPA1, LPA2 and LPA3 in cervical cancer. LPA2 and LPA3 are crucial for *in vivo* tumor growth through Gi/PI3K/AKT, Gi/PKC and IκB/NF-κB signaling, in scid mice [21].

Notch Signaling Mediated-PI3K Pathway

Notch signaling is important for cellular differentiation and proliferation, which complements the function of papillomavirus oncogenes in transforming human

keratinocytes. Notch signaling mediates several signaling pathways in the majority of human cervical cancers. There is a complex interplay between Notch signaling and papillomaviruses in the context of cervical carcinogenesis [22]. The Notch signaling operates two pro-oncogenic effector mechanisms: 1). activation of PI3K/AKT pathway and 2). upregulation of c-Myc. According to the analysis of expression of Notch1, Jagged 1, Hes1, pAKT, NF-κB p50, NF-κB p65, IκB-α, Bcl-2, CyclinD1, Cdk9, c-Fos, and p53 in 352 biopsies that included 69 normal cervical tissue, 132 preinvasive lesions and 151 squamous cell carcinomas of the uterine cervix, induction of PI3K by Notch signaling in human cervical tumors preferentially upregulates Jagged1 expression [23]. Jagged1 expression correlates with the rapid induction of PI3K-mediated epithelial-mesenchymal transition (EMT) [24]. Also, there is a co-activation of Notch1 and NF-κB signaling pathways at the cellular level in the majority of human cervical cancers [23]. Thus, strategies to modulate Notch-PI3K signaling may facilitate therapeutic intervention [25].

AKT/mTOR Signalings

Activations of AKT/mTOR are frequently observed in cervical squamous cell carcinoma [26]. In a clinical study, a tissue microarray analysis has shown that the AKT/mTOR pathway is activated in cervical carcinomas. 12 out of 25 cervical cancer patients at stage Ib2-IIb (48%) showed AKT activation in the cytoplasm and nucleus of the cancer cells while the other 13 patients (52%) expressed phosphorylated mTOR (p-mTOR) in the cytoplasm and membrane of the cancer cells [26]. Both activated AKT and mTOR are found to be significant prognostic indicators. Moreover, expression of p-mTOR and distant metastasis significantly correlate with the response to nucleus accumbens core (NAC). Post NAC evaluation of the primary tumor reveals 17/25 (68%) responsive tumors [26]. Tissue microarray analysis in another clinical study has revealed that expression of EGFR; mTOR pathway markers, p-mTOR$^{(Ser2448)}$ and p-p70S6K$^{(Thr389)}$ varies in 20 normal cervix and 60 cases of NL, HSIL and SCC cervical biopsies and cervical conizations [27]. Plasmalemmal EGFR expression is limited to the basal/parabasal cells in normal cervical epithelium, but diffusely positive in all HSIL/SCC. The pattern of cytoplasmic p-mTOR and nuclear p-p70S6K expression is similar to that of EGFR. Nuclear translocation of p-mTOR in all SCC lesions is significantly increased versus both HSIL/NL [27]. Thus, expression of p-mTOR can serve as as an independent poor prognostic marker to predict response to chemotherapy and survival of cervical cancer patients [26].

TARGETING PI3K SIGNALLING PATHWAY FOR CERVICAL CANCER THERAPY

Three treatment methods are commonly used for cervical cancer therapy: surgery, chemotherapy and radiotherapy. The three methods can be used alone, but they are used in conjunction with one another most of the time. Early stage cervical cancer is treated with surgery or radiation with equivalent results [28]. Radiotherapy may be given alone or before surgery to shrink the tumor. Cervical cancer patients are often treated with chemotherapy, a treatment method that uses anticancer drugs to kill cancer cells. But many cervical cancer patients are treated with a combination of chemo-therapy and radiotherapy, which is called as chemo-radiotherapy a mainstay for anti-tumor therapeutic regimens for a variety of tumors. The concurrent chemoradiotherapy of locoregionally advanced cervical cancer dramatically improved the local control and overall survival compared with the traditional radiotherapy [29]. Chemoradiotherapy triggers the tumor cell apoptotic pathways by either directly eliciting DNA damage or indirectly inducing the formation of oxygen radicals [30]. Thus, evolving understanding of the molecular events of chemoradiotherapy in this cancer is very important, which can uncover a host of promising targets for the molecular mechanism-based therapy. Among the several molecular pathways reported, the PI3K/AKT signaling pathway accounts for the major mechanism of the chemoradiotherapy.

Growth Factors/PI3K/AKT-mediated Chemoradiotherapy

Chemoradiotherapy is deemed the standard treatment for treating the locally advanced cervical cancer. Favorable long-term outcomes have been reported for patients with good responses to chemoradiotherapy. Thus, predictive molecular events for chemoradiotherapy responses can be useful for their applicability to risk-adaptive therapy in cervical cancer patients. Based on the ongoing clinical trials, the PI3K/AKT pathway regulated by the EGFRs is a promising therapeutic target [31]. Noordhuis and colleagues determined the relation between proteins involved in the EGFR pathway and response to chemo-radiotherapy and survival in a large, well-documented series of cervical cancer patients [32]. Tissue samples of 375 consecutive stages Ib to IVa cervical cancer patients treated with chemo-radiotherapy between January 1980 and December 2006 were assessed by immunohistochemistry on tissue microarrays. Both EGFR (35.3%) and pEGFR (19.7%) immunostainings are frequently observed and independently associated with poor response to therapy and disease-specific survival in cervical cancer patients primarily treated by chemo-radiation. Cytoplasmic staining of pEGFR is independent predictors of poor response to chemo-radiation. Membranous EGFR staining also is an independent prognostic factor for poor disease-specific survival [31]. In another clinical trail, Lee and colleagues investigated prognostic significance of tumor expression of different biomarkers such as HER1 (EGFR), HER2 (c-erb-B2), HER3 (c-erb-B3), HER4 (c-erb-B4) and p-AKT and their correlations in cervical carcinoma patients treated with radiation [33]. Fifty-five patients with stages I-IVA cervical carcinoma were treated with definitive radiotherapy. They observed that decreased expressions of HER2, HER4 and p-AKT were significant for diminished disease-free survival while increased EGFR, and diminished HER2, HER4 and p-AKT expression were significant or showed trends toward significance for diminished overall survival [33].

Cell biological markers have been used to review the prognostic and predictive significance in a clinical study of 50 cervical cancer patients with chemoradiotherapy [32]. In addition to cyclooxygenase-2 (COX-2) and serum squamous cell carcinoma antigen (SCC-ag) levels, markers associated with poor prognosis were involved in EGFR and C-erbB-2 signalings and in angiogenesis and hypoxia (carbonic anhydrase 9 and hypoxia-inducible factor-1alpha). Both

EGFR and C-erbB-2 were also associated with poor response to chemoradiotherapy. EGFR signaling is associated with poor prognosis and response to therapy in cervical cancer patients primarily treated with chemoradiation, whereas markers involved in angiogenesis and hypoxia, COX-2, and serum SCC-ag levels are associated with a poor prognosis [32]. Furthermore, the prognostic value of the 4E-BP1 activation state and related upstream/downstream signaling proteins on the clinical outcome of patients with intermediate- or high-risk early-stage cervical carcinoma treated with postoperative radiotherapy have been investigated to determine the optimal treatment of early-stage cervical carcinoma [34]. In another clinical study, immunohisto-chemical staining was performed on 64 cervical carcinoma surgical specimens for each protein of the panel (p4E-BP1, p-MAKP, p-AKT, VEGF, KDR, Bcl-2, TP53, receptor for activated C-kinase 1. All patients received postoperative radiotherapy. Concurrent chemotherapy was added if high-risk features were present. The median follow-up was 40 months. Of the 64 patients, 13 received concomitant chemotherapy. p4E-BP1 overexpression in moderate/high-risk early-stage cervical carcinoma correlated significantly with disease-free survival (hazard ratio, 4.39) and overall survival (hazard ratio, 4.88). VEGF and its receptor KDR had positive immunoreactivity in all tumor samples. Moderate/high-risk early-stage cervical carcinoma with low p4E-BP1 expression was highly curable with the current postoperative treatments [34]. In addition, cervical tumor response on post-therapy 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) is predictive of survival outcome. In a recently published clinical study, Schwarz and colleagues reported gene expression profiling in 62 pre-treatment biopsies from advanced cervical cancer patients [35]. Patients were treated with definitive radiation. Fifty-three patients received concurrent chemotherapy. All patients underwent a pre-treatment and a 3-month post therapy of FDG-PET/CT. 40 patients showed a complete metabolic response (PET negative group) and 22 incomplete metabolic response (PET positive group). The 3-year cause-specific survival estimates were 98% for the PET negative group and 39% for the PET positive group. GSEA identified alterations in expression of genes associated with the PI3K/AKT signaling pathway in patients with a positive PET. Immunohistochemical analysis of tissue microarray of 174 pre-treatment biopsies confirmed that p-AKT is a biomarker for poor prognosis in cervical cancer. Radiation induced AKT mediated cytoprotective effect was countered by Fused Toes Homolog (FTS) knockdown which leads to PARP cleavage and caspase-3 activation leading to cell death [36]. Activation of the EGFR signaling pathway has been reported to induce resistance to chemoradiotherapy in cancers, such as head and neck cancer, whereas EGFR-targeted agents in combination with chemoradiotherapy seem to improve treatment efficacy. Thus, it is clear that targeting the growth factor/PI3K/AKT pathways may improve survival in advanced-stage cervical cancer patients treated with a combination of chemoradiation [32].

PI3K/AKT/mTOR Signaling-mediated Chemoradiotherapy

Extensive *in vitro* experiments have suggested that chemoradiotherapy induces activation of PI3K that in turn activates multiple signaling pathways to play critical roles in determining cell fate in cervical cancer cells. For example, aspirin has therapeutic value in cervical cancer by inducing apoptosis and inhibition of proliferation in HeLa cells, due to proteosome-mediated degradation of oncogene ErbB2 protein to suppress its amplification and expression and inhibition of ERK/AKT [37]. ZSTK474 a specific PI3K inhibitor inhibits tumour cell proliferation *via* G1 arrest of the cell cycle *in vitro* but did not increase the subdiploid cells or activate caspase, both of which are hallmarks of apoptosis [38]. In a animal preclinical study, ZSTK474 effectively inhibited the growth of human cancer xenografts *in vivo* by suppressing expression of p-AKT, nuclear cyclin D1 and Ki67 hallmarks of proliferation, did not increase TUNEL-positive apoptotic cells [39]. Activation of the PI3K/AKT pathway is associated with incomplete metabolic response in cervical cancer. Targeted inhibition of PI3K/AKT may improve response to chemoradiation [35]. Naringenin itself exhibited no anti-cancer activity, but a synthesized naringenin derivative N101-2 inhibited growth of the cervical cancer cells by increasing expression of p53, cyclins, pRb and Fas/FasL, activating Fas-mediated extrinsic apoptosis signaling. N101-2 induces apoptosis by arresting the cell cycle at sub-G1 phase accompanied by inhibiting the PI3K/AKT pathway to up-regulate the tumor suppressor PTEN and its upstream regulator PPARγ in CaSki and SiHa cervical cancer cells [40]. However, ionizing radiation triggers Bax and Bak activation, Bcl-2 down-regulation, and subsequent mitochondrial cell death. Inhibition of PI3K effectively attenuates radiation-induced mitochondrial cell death and increases clonogenic survival. Inhibition of PI3K also suppresses SEK-1/MKK-4 and JNK activation, Bax and Bak activation, and Bcl-2 down-regulation. In contrast, inhibition of p38 MAPK led to enhanced Bax and Bak activation and mitochondrial cell death. Thus, the PI3K-SEK-1/MKK-4-JNK pathway is required for the mitochondrial-mediated cell death in response to radiation, whereas the c-Src-Rac1-p38 MAPK pathway plays a cytoprotective role against mitochondrial-mediated cell death [41].

Recently, the antitumor effects of several PI3K pathway inhibitors such as 3,3-diindolylmethane (DIM), S-1 (an oral fluoropyrimidine), capecitabine and cisplatin have been evaluated in both *in vitro* experiments and clinical trails for the cervical cancer therapy [42-46]. These drugs strongly inhibited proliferation and induced apoptosis of the cervical cancer cells HeLa and SiHa by significantly suppressing expression of PI3K, AKT and other molecules involved in the PI3K pathway [43,45]. In a pragmatic double-blind, randomised controlled trial of 150 mg DIM or placebo daily for 6 months in women with newly diagnosed, low-grade cytological abnormalities [42]. Of the 551 randomised women available for analysis, 9% on DIM and 12% on placebo had cervical intraepithelial neoplasia-2 (CIN2) or worse after 6-month supplementation (risk ratio (RR) 0.7 (95% confidence interval (CI):0.4-1.2)), whereas 4.6% and 5.1%, respectively, had CIN3 or worse (RR 0.9 (95% CI:0.4-2.0)). A total of 27.3% of women on DIM and 34.3% on placebo had no sign of disease (negative cytology, colposcopy and HPV tests) at 6 months (RR 0.8 (95% CI: 0.6-1.0)). Clearly, short-term DIM supplementation (150 mg

day^{-1}) is well tolerated, suggesting that it could potentially halt cervical carcinogenesis although uncertainty remains regarding its effect on CIN^{2+} [42]. A phase II trial using capecitabine and cisplatin was carried out in 22 women (median age was 51 years, ranging from 37-70 years) with advanced, persistent, or recurrent cervical carcinoma between November 2004 and October 2007 [46]. Seventeen patients had prior radiotherapy, and 13 received a radiation sensitizer, whereas 2 patients underwent surgery exclusively and 3 patients had no prior treatment. Treatment consisted of 50 mg/m of intravenous cisplatin on day 1 with 2500 mg/m oral capecitabine daily in 2 divided doses for 14 consecutive days in 21-day cycles [46]. This trail suggested that the capecitabine-cisplatin combination is a moderately tolerated and active regimen in advanced, persistent, or recurrent cervical carcinoma patients [46].

Both basic and preclinical studies have shown that the mTOR inhibitor, rapamycin, and EGFR-tyrosine kinase inhibitor, erlotinib, can induce growth delay of xenografts using HPV-containing human cervical carcinoma cell lines [27], suggesting that the mTOR cascade may be a promising target for therapeutic intervention in cervical cancer [26]. mTOR-specific siRNA effectively suppresses growth of HeLa cells through mechanisms including inhibition of the cell cycle and increased apoptosis [27]. According to the precilical and clinical studies, inhibition of mTOR represents a potential therapeutic strategy for cervical cancers [47,48]. The PI3KCA-mutated cancer patients treated with PI3K/AKT/mTOR inhibitors exhibit a higher response rate than patients without mutations, thus mutations of the PI3KCA gene may predict response to PI3K/AKT/mTOR inhibitors [48]. PI3K inhibitor LY294002 efficiently inhibited HeLa cell growth with IC50 of 20.77 μM, and induced apoptosis up to 36% at 3h post-treatment. Thus, the novel targeted therapies for the PI3K/AKT/mTOR signaling pathway components can provide a useful adjuvant therapeutic strategy for cervical cancer [49]. Rapamycin inhibits growth of all the cervical cancer cell lines examined in a dose-dependent manner with IC50 values <50 nM while it induces G1 arrest in those cell lines sensitive to its growth inhibitory effects. Furthermore, rapamycin rapidly inhibits phosphorylation of S6 and results in decreased levels of total S6 protein. Apparently, rapamycin may potentially exert its anti-tumor effects through two independent pathways by G1 cell cycle arrest as well as suppression of telomerase activity by inhibition of hTERT mRNA transcription[50].

Targeting PI3K Signalling Pathways to Overcome Chemoradiotherapy Resistance

According to the published clinical studies, cervical cancer in some cases is radioresistant although radiotherapy is the major treatment modality for this disease sometimes [36]. Basic research has provided evidence that constitutive AKT levels are the lowest in cell lines that are the most resistant to cisplatin (CDDP), 5-FU, and radiation [51] and radiation induces AKT-mediated cytoprotective effect [36]. Although radiation and CDDP and 5-FU result in decreased survival of HPV-positive cervical cancer cells, HPV-negative cervical cancer cell C33A is more sensitive to radiation than the other cell lines. It has been demonstrated that HPV16- and 18-positive CaSki and SiHa cells are the most resistant to the therapeutic drugs such as CDDP and 5-FU and radiation treatments. Anandharaj and coleagues evaluated the role of Fused Toes Homolog (FTS) in radiation resistance of cervical carcinoma and observed that FTS localization and expression are changed in cervical cancer cells and tissues after radiation. Targeted stable knockdown of FTS in HeLa cells leads to the growth inhibition after radiation. AKT-mediated cytoprotective effect induced by radiation is countered by FTS knockdown which leads to PARP cleavage and caspase-3 activation leading to cell death. FTS knockdown promotes radiation induced cell cycle arrest at G0/G1 and apoptosis of HeLa cells. Thus, FTS is involved in radioresistance of cervical cancer. Targeted inhibition of FTS leads to the shutdown of key elemental characteristics of cervical cancer and can be an effective therapeutic strategy [36].

Based on the published basic and clinical studies, the radiation-activated PI3K/AKT/COX-2 signal transduction pathway is the main cause for decline in radiosensitivity in cervical cancer cells [52,53]. Thus, defining the molecular events that contribute to chemoradiotherapy resistance and progression of cancer is very important. The PI3K/AKT signaling leads to radiation resistance and pAKT is a major contributor to radioresistance in human cervical cancers. In a published clinical study, 27 women were received with primary radiation therapy due to locally advanced cervical cancer (LACC) with FIGO stage IIB-IVA. Eighteen patients did not show local recurrences regarded as a radiation-sensitive group. Nine patients regarded as radiation resistant developed local recurrences with a median progression free interval of 9 months [53]. Immunohistochemical analysis of the biopsies from all the 27 patients revealed that pAKT expression is significantly associated with local recurrence, which is significantly more frequent in the radiation-resistant group than in the radiation-sensitive group. The mean progression-free survival is 86 months for patients with pAKT-negative staining (19 cases) and 44 months for patients with pAKT-positive expression (eight cases) [53].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis and kills cancer cells with little or no adverse effects on normal cells. TRAIL is relatively safe for clinical applications [54]. However, TRAIL resistance is widely found in cancer cells leading to limitations in utilizing TRAIL as a therapeutic agent for cancer treatment. Recently, artesunate, an effective and safe anti-malarial drug, is also described as a promising candidate for cancer therapy. Artesunate effectively enhances TRAIL-mediated cytotoxicity by suppressing pro-survival proteins, such as survivin, XIAP and Bcl-XL. Upon treatment with artesunate, the levels of survival proteins are strongly suppressed in HeLa cells. The down-regulation of these survival proteins can be regulated by repressing activation of NF-κB and AKT. Artesunate also inhibits TRAIL-induced transcriptional activity of NF-κB. This substance significantly enhances both extrinsic and intrinsic apoptosis, which are induced by TRAIL. Thus, artesunate can overcome TRAIL resistance and treatment of TRAIL combined with artesunate may be an effective strategy for cervical cancer therapy [54]. Furthermore, Grisendi and colleagues reported that adipose-derived mesenchymal stromal/stem cells (AD-MSC) transduced with a retroviral

vector encoding full-length human TRAIL could induce apoptosis in a variety of human cancers including human cervical carcinoma but not normal tissues. When the AD-MSC armed with TRAIL that were injected i.v. or s.c. into mice, AD-MSC armed with TRAIL localized into tumors and mediated apoptosis without significant apparent toxicities to normal tissues, which provided a preclinical support for a model of TRAIL-based cancer therapy relying on the use of adipose-derived mesenchymal progenitors as cellular vectors [55]. Recently, in an animal preclinical trail, the mice bearing xenograft cervical tumors showed significantly reduced tumor growth and enhanced tumor survival after they were intratumorally administrated with early growth response-1 (Egr-1)/TRAIL adenovirus followed by radiation [56]. Thus, it appears that the Egr-1/TRAIL adenoviral gene product may offer a potential "one-two punch" tumor therapy for cervical cancers by potentiating radiation treatment.

PI3K Inhibitors Sensitize Cervical Cancer Cells to Chemoradiotherapy

Chemoradiotherapy is used for curative therapy of locally advanced cancers that combines radiation with additional anti-tumor agents to improve the therapeutic efficacy. Clonogenic assays (CA) reveals that cetuximab with cisplatin and radiation achieved maximum cytotoxic effects for A431, Caski and C33A cells (high, intermediate and low EGFR expression, respectively) [57]. Cetuximab efficiently decreases MAPK and AKT phosphorylation in A431 cells but slightly less in Caski and C33A cells. To check whether further EGFR, HER2 or MAPK inhibition would improve cetuximab's cytotoxicity, Meira and coleagues combined cetuximab with an EGFR tyrosine kinase inhibitor (TKI), trastuzumab or a MEK1/2 inhibitor (PD98059) to treat different cervical cancer cells. In Caski, but not in C33A cells, cetuximab cooperates with the TKI to reduce cell survival and AKT and MAPK phosphorylation. Cetuximab combined with chemoradiation, trastuzumab or MAPK inhibitors has useful applications for CC treatment [57]. Kim *et al*. generated a double E1B 19 kDa- and E1B 55 kDa-deleted oncolytic adenovirus (Ad-DeltaE1B19/55) to augment radiation therapy. In combination with radiotherapy, greater cytotoxicity was observed for Ad-DeltaE1B19/55 than for the single E1B 55 kDa-deleted oncolytic Ad. Higher levels of p53, p-p53, p-Chk1, p-Chk2, PI3K, p-AKT, cytochrome c, and cleavage of PARP (poly (ADP-ribose) polymerase) and caspase-3 are expressed in cells treated with Ad-DeltaE1B19/55 compared with those treated with Ad-DeltaE1B55, indicating that the E1B 19 kDa present in Ad-DeltaE1B55 may partially block radiation-induced apoptosis. Tumors treated with Ad-DeltaE1B19/55 and radiation showed large areas of necrosis and apoptosis with the corresponding induction of p53. In an *in vivo* animal experiment, the combination of Ad-DeltaE1B19/55 and radiation is more efficacious than the combination of Ad-DeltaE1B55 and radiation [30].

Furthermore, inorganic arsenic is a major environmental contaminant associated with an increased risk of human skin cancer [58]. Arsenic trioxide (As2O3)-induced apoptosis has been elucidated extensively in hematologic cancers. As2O3 triggers apoptosis and significantly downregulates stathmin expression in cervical cancer cells through the PI3K signaling pathway. Expression of a siRNA targeting stathmin enhances As2O3-triggered apoptosis in cell culture and in mouse models. As2O3 as a therapeutic agent through combination treatment with stathmin inhibition or PI3K/AKT inhibitors can improve the therapeutic efficacy [59]. It has been observed that arsenic leads to an increase in p53 and its binding to DNA in two epithelial cell types: primary normal human keratinocyte cultures (NHK) and the carcinoma-derived C33-A cell line, but the final outcome was different. In NHK, arsenic triggers a sustained activation of the PI3K/AKT/GSK3β pathway, driving the cell into a cell-differentiated stage in which the proliferation signals are turned down. In contrast, arsenic leads to a transient increase in p53 followed by a drastic reduction in its nuclear levels and an increase in cell proliferation in C33-A cells. These findings favor the notion that p53-stage and transcriptional abilities are important to understand modifications in the proliferation-differentiation balance, an equilibrium that is severely impaired by arsenic [58].

PHYTOCHEMICALS AS THE POTENTIAL THERAPEUTICS FOR CERVICAL CANCERS

Phytochemicals are the natural chemical compounds derived from plant, fruit, bacterium and fungi. Abundant evidence from epidemiological studies has indicated that the phytochemicals have been used to significantly reduce the risk of cancers [60]. As an emerging strategy, the phyto-chemicals can be used not only to prevent, impede and delay cancer, but also to cure cancer. In an animal preclinical study, Jin *et al*. reported that the antiestrogenic phytochemical indole-3-carbinol (I3C) is a useful preventive for cervical-vaginal cancer in mice that express HPV16 transgenes under a keratin 14 promoter (K14-HPV16 mice) [61]. I3C also reduced dysplasia in the nontransgenic mice and appeared to reduce skin cancer in transgenic mice [61]. Several phytochemicals have been used as therapeutics for cervical cancer treatment such as LY294002 (a morpholine derivative of quercetin) and Taxol (paclitaxel) [62,63]. In a phase II trial, twenty-nine patients with stages IB2 to IVa cervical cancer were treated with 40 mg/m intravenous (i.v.) cisplatin per week and 50 mg/m i.v. paclitaxel combined with 45 Gy of pelvic external beam radiation therapy per week and 30 Gy of high-dose-rate brachytherapy [64]. After a median follow-up of 48 months, 7 patients (24.1%) experienced severe (Radiation Therapy Oncology Group grade 3 or higher) late toxicity. No fatal events were observed. Seven patients failed, 1 locally and 6 at distant sites. The 8-year local/pelvic control rate was 95.7%, and the 8-year freedom from systemic failure rate was 76.1%. Eight-year actuarial disease-free survival and overall survival were 63.1% and 75.9%, respectively. However, this study demonstrated unacceptable toxicity of combining the stated doses of concurrent cisplatin and paclitaxel chemotherapy with definitive radiotherapy for patients with advanced cervical cancer. Thus, It was recommended that additional phase I/II trials are required to clearly establish the recommended phase II dose for these drugs [64]. Other phytochemicals are being tested as the potential therapeutics for cervical cancer. The potential phytochemicals include

amentoflavone [65], ascochlorin [66], boswellic acid [67, 68], curcumin [69], genistein [70], green tea extracts [71]; oridonin [72]; silibinin [73, 74], silymarin [73, 74], resveratrol (trans 3,5,4'-trihydroxystilbene) [75] and triptolide [70] etc. Thus, the anticancer properties and mechanisms of these phytochemicals in both *in vitro* experiments and preclinical trail studies are discussed below.

Phytochemicals-induced Cytotoxicity

All the reported phytochemicals have shown to inhibit proliferation of the cancer cells and induce apoptosis. Paclitaxel is the best anticancer agent isolated from plants, which inhabits cell proliferation of cervical cancer cell lines (HeLa and CaSki) and induces apoptosis [62]. HeLa cells have a much higher sensitivity to paclitaxel, while CaSki cells show a lower sensitivity [76,77]. Oridonin, a diterpenoid isolated from Rabdosia rubescens, suppresses cell proliferation and induces apoptosis of cervical cancer cells [72]. Resveratrol exhibits anticancer properties by suppressing proliferation of a wide variety of tumor cells including cervical carcinoma [75]. A pentacyclic triterpenediol (TPD) from Boswellia serrata induces apoptotic cell death of both HeLa and SiHa cells [78]. Genistein (GEN), a naturally occurring flavonoid present in soy bean, inhibits growth of HeLa and CaSki cells [70]. Phytochemicals present in tea, particularly polyphenols such as green tea polyphenol (-)-epigallocatechin gallate (EGCG) and black tea polyphenol theaflavins (TF) inhibit dramatically growth and proliferation of HeLa cells [79]. Amentoflavone, a biflavonoid from Selaginella tamariscina, is known to possess several bioactivities. Amentoflavone induces apoptosis in SiHa and CaSki cells by suppressing HPV E7 expression, showing its antitumor property [65]. Silymarin the seeds of the milk thistle plant is widely used as a hepatic protection agent due to its antioxidant-like activity. Treatment of cervical cancer cells (C-33A) with silymarin results in a significant decrease in cell viability [74]. Curcuma wenyujin extract (CWE) as an essential oil extract that derived from the rhizome of CW inhibits tumor growth and suppresses colony formation by inhibiting the proliferation of HeLa cells [80].

It is clear that the phytochemicals arrest cell cycle to produce inhibitory effects and induce apoptosis in the treated cancer cells**.** Treatments of several phytochemicals such as paclitaxel and deoxypodophyllotoxin (DPPT) result in significant G2/M phase arrest [77, 81] while other phytochemicals such as biflavonoid amentoflavone, EGCG, TF and CWE have been reported to arrest the cancer cells at G1 phase. All the phytochemicals induce cell-cycle arrest followed by apoptosis through multiple cellular processes. DPPT inhibits cell viability resulting from G(2)/M phase cell cycle arrest, accompanied by an increase in apoptotic cell death due to morphologic changes and internucleosomal DNA fragmentation and inhibition of tubulin polymerization [81]. Furthermore, DPPT treatment results in the activation of ATM, upregulation of p53 and Bax, activation of caspase-3 and -7, and accumulation of PTEN [81]. Both EGCG and TF induce G1 phase arrest by attenuating mitochondrial membrane potential with the increase of reactive oxygen species generation, p53 expression, Bax/Bcl-2 ratio, cytochrome-c release, and cleavage of procaspase-3 and -9 and poly(ADP-ribose)-polymerase [79]. Silymarin also suppresses C-33A cell invasion and wound-healing migration in a concentration-dependent manner and significantly inhibited the expression of matrix metalloproteinase-9 (MMP-9) [74]. Amentoflavone activates PPARγ/PTEN expressions to induce apoptosis *via* suppressing HPV E7 expression, arresting cell cycle at G_1 phase in both SiHa and CaSki cells [65]. CWE-treated cells display G1 arrest and apoptosis accompanied with the reduction of PTEN, AKT and STAT3 phosphorylation and downregulation of NF-κB signaling [80]. Moreover, CWE inhibits tumor growth in a xenograft mouse tumor model injected with Hela cells, by inhibiting cell proliferation and inducing apoptosis [80]. The growth-inhibitory effects of resveratrol are mediated through cell-cycle arrest; upregulation of p21Cip1/WAF1, p53 and Bax; down-regulation of survivin, cyclin D1, cyclin E, Bcl-2, Bcl-xL and clAPs; and activation of caspases [75].

Phytochemicals-targeted PI3K Signalling Pathway

Growth Factors- Upstream Regulator of PI3k/AKT Pathway

As discussed above, growth factors including EGF, HIF-1 and VEGF act as the upstream regulators of the PI3k/AKT signalings to regulate the cellular proliferation, differentiation, metabolism, survival complex and cancer progression. It is well known that EGFR activation is absolutely required for proliferation of cervical cancer cell. Published studies have provided evidence that several phytochemicals prevent cancer progression, inhibit cell growth and induce apoptosis in cervical cancers by up-regulating or down-regulating expression and activation of different growth factors and their receptors. The green tea polyphenol EGCG treatment induced apoptotic cell death of the cervical cancer cells by inhibiting EGF-dependent activation of EGFR, EGFR-dependent activation of the MAPK-ERK1/2 and EGFR-dependent AKT activity [82]. But, EGCG does not affect the EGFR-dependent activation of JNK, indicating that its inhibitory role in cervical cancer is selective [82]. Ascochlorin functionally abrogates *in vivo* tumor angio-genesis induced by EGF [66]. Paclitaxel is classified as a plant alkaloid. As an anticancer chemotherapy drug, paclitaxel has been used to treat many types of solid tumor cancers including cervical cancer [83,84]. In an *in vitro* experiment, SiHa cells exposed to paclitaxel undergo apoptosis, but EGF strongly prevented this apoptosis. The EGF-prevented apoptosis is not altered by pharmacological blockade of PI3K with the PI3K specific inhibitor LY294002 or blockade of the MAPK/MEK with the MEK specific inhibitor PD98059 [85]. This is due to that EGF activates only the JNK activity, not the PI-3K/AKT, MEK/MAPK, or p38 MAPK activity in SiHa cells. The findings suggest that the JNK signaling pathway plays an important role in EGF-mediated protection from paclitaxel-induced apoptosis in SiHa cells [85].

HIF-1 plays a critical role for tumour adaptation to microenvironmental hypoxia, and represents an appealing chemotherapeutic target. Silibinin a nontoxic flavonoid extracted from the milk thistle seeds exhibits anticancer properties by inhibiting hypoxia-induced HIF-1α accumulation and HIF-1 transcriptional activity in HeLa cells [73]. Recently, Ascochlorin, a non-toxic prenylphenol compound,

has been shown to have strong anti-cancer effects on various human cancer cells [66]. This non-toxic prenylphenol compound selectively inhibits HIF-1α expression in response to EGF stimulation, but not in response to hypoxia or treatment with a transition metal (CoCl(2)) in CaSki cells [66]. Interestingly, Ascochlorin inhibited EGF-induced ERK-1/2 activation but not AKT activation although both play essential roles in EGF-induced HIF-1α protein synthesis [66]. Targeted inhibition of EGFR expression using an EGFR-specific siRNA confirms that ascochlorin inhibits HIF-1α expression through suppression of EGFR activation [66].

VEGF a downstream target of HIF-1α plays a critical role in tumor angiogenesis function as therapeutic target for green tea extract and EGCG in the context of cancer chemoprevention and anticancer therapy [71]. Silibinin reduces hypoxia-induced VEGF release in both HeLa and Hep3B cells [73] and this effect is potentiated by the PI3K/AKT inhibitor LY294002. Amentoflavone up-regulates expression of PPARγ and PTEN proteins by inhibiting E7-mediated COX-2/IL-32 expressions [65]. Green tea extract and its major component EGCG exhibit antiangiogenic activities in various experimental tumor models. Green tea extract and EGCG significantly inhibit hypoxia- and serum-induced HIF-1α protein accumulation in these cancer cells but had no effects on HIF-1α mRNA expression. Suppression of HIF-1α protein by green tea extract and EGCG also resulted in a drastic decrease in VEGF expression. The mechanisms of green tea extract and EGCG inhibition of hypoxia-induced HIF-1α protein accumulation seem to involve the blockage of both PI3K/AKT and ERK 1/2 signaling pathways and the enhancing of HIF-1α protein degradation through the proteasome system [71].

AKT/mTOR Signaling Cascades

Extensive research has shown that most of the phytochemicals target directly many molecules which are involved in PI3k/AKT signalling pathway**.** Human Cripto-1 (CR-1), a member of the epidermal growth factor-CFC peptide family, may function as a survival factor by activating the ras/raf/MAP/ERK/MAPK through a PI3K - dependent signaling pathway involving PI3K/AKT/GSK-3β [86]. In SiHa cells, CR-1 can enhance tyrosine phosphorylation of the p85 regulatory subunit of PI3K and transiently induce phosphorylations of AKT and GSK-3β. LY294002 the well known inhibitor of PI3K blocks the CR-1-induced phosphorylations of AKT and GSK-3β leading to apoptosis [86]. Paclitaxel downregulates phosphorylation of AKT and dramatically diminishes the transactivation of TACC3 promoter to downregulate TACC3 protein expression in cervical cancer cells in both HeLa and CaSki cells [77]. Tea polyphenols (EGCG and TF) inhibit proliferation of cervical cancer cells by inducing apoptosis and inhibiting activation of AKT and NF-κB and leading subsequently to degradation of inhibitor of κBα and κBβ subunits, thereby downregulating cyclooxygenase-2 [79]. EGCG acts simultaneously at multiple levels to inhibit EGF-dependent signalling by directly inhibiting ERK1/2 and AKT [82]. A pentacyclic triterpenediol (TPD) robustly up-regulates time-dependent expression of p53/p21/PUMA to abrogate PI3K/AKT pathways and cause oxidative stress by early generation of nitric oxide and reactive oxygen species in cervical cancer cells [78]. TPD also decreases the expression of PI3K/pAKT, ERK1/2 and NF-κB/AKT signaling cascades [78]. Triptolide-induced apoptosis is associated with a marked reduction in AKT phosphorylation [70]. The other phytochemicals including resveratrol, boswellic acid (BCDD), genistein, oridonin, diterpenoid, amentoflavone, curcumin and silymarin have also been reported to inhibit proliferation of cervical cancers by preventing phosphorylation of AKT the major survival factors of the cancer cells [87]. But these phytochemicals also show to act on different molecules involved in PI3K pathway. For example, boswellic acid (BCDD) robustly up-regulates time-dependent expression of p53/PUMA/p21, whereas it deprives the essential p-AKT and NF-κB cell survival signalling cascade [87]. Genistein induces phosphorylation of p38 MAPK and JNK [88]. Oridonin constitutively suppresses activated FOXO and GSK3 beside AKT in HeLa cells [72]. Diterpenoid downregulates expression of forkhead box class O (FOXO) transcription factor and glycogen synthase kinase 3 (GSK3) and induces the release of cytochrome c accompanied by the activation of caspase-3 and poly-adenosine diphosphate-ribose polymerase cleavage [72]. Curcumin downregulates specific genetic targets including NF-κB, STAT3, COX2, antiapoptotic proteins, growth factor receptors, and multidrug-resistance proteins [89]. Silymarin upregulates expression of phosphatase and PTEN [74]. Resveratrol has been shown to suppress the activation of several transcription factors, including NF-κB, AP-1 and Egr-1 to inhibit protein kinases including IκBα kinase, JNK, MAPK, PKC, PKD and casein kinase II and to downregulate expression of genes such as COX-2, 5-LOX, VEGF, IL-1, IL-6, IL-8, AR and PSA [75].

mTOR acts both as a downstream effector and upstream regulator of PI3K. Alterations of the AKT/mTOR pathway have been observed in numerous types of cancer. p-AKT and p-mTOR were identified in 50% and 53.8% of adenocarcinoma of the cervix [73]. Expression of p-mTOR is a significant prognostic marker of adenocarcinoma of the cervix, which is a potential molecular target for the treatment of cervical cancer [68]. Inhibition of the AKT/mTOR pathway by phytochemicals such as LY294002 contributes to cisplatin-induced apoptosis in both patients with cervical adenocarcinoma *in vivo* and cervical cancer cell lines *in vitro* [73]. Silibinin, known as silybin, is the major active constituent of silymarin, a standardized extract of the milk thistle seeds, which contains mixture of flavonolignans. Silibinin shows its anticancer activity by inhibiting cancer cell proliferation through the AKT/mTOR pathway [73]. In addition, pre-treatment with rapamycin inhibits activation of mTOR signalling and significantly enhances the sensitivity of CaSki cells to paclitaxel by increasing apoptotic cell death partly through caspase activation. It is clear that paclitaxel exerts its anti-tumour effects on cervical cancer cells by inducing apoptosis through intrinsic pathway, and rapamycin targeted to mTOR can sensitise paclitaxel-resistant cervical cancer cells [90].

Caspase-cascade

Several phytochemicals have been reported to act with caspase-cascade (caspases) to exert their anti-cancer

properties in *in vitro* cervical cancer-cell experiments. Caspases are a family of cysteine proteases that play the essential roles in apoptosis, necrosis, and inflammation. CWE treatment results in apoptosis in HeLa cells through caspase activation and PARP cleavage, which can be reversed by a pan-caspase inhibitor. Triptolide is the main active component of the traditional Chinese herbal medicine Tripterygium wilfordii Hook F. Triptolide treatment results in loss of mitochondrial membrane potential, caspase activition (caspase-8, -9 and -3) and cleavage of the caspase substrate, poly (ADP-ribose) polymerase to induce apoptotic cell death [70]. DPPT activates caspase-3 and -7 to induce cancer cell apoptosis accompanied with up-regulated expression of tumor suppressors (p53, Bax and PTEN), activation of DNA damage-sensing kinases (ataxia-telangiectasia mutated (ATM) kinase and Chk2) and inhibition of the AKT pathway [81]. Amentoflavone treatment activates caspase-3 and -9 and results in proteolytic cleavage of poly-(ADP-ribose) polymerase to induce apoptosis of the cervical cancer cells by increasing expression of the apoptotic factor Bax and decreasing expression of the anti-apoptotic factor Bcl-2 [65]. Recently, Yu and colleagues report that silymarin induces the apoptotic death of cervical cancer cells through the modulation of Bcl-2 family proteins and activation of caspase 3 [74].

Phytochemicals Used as Chemoradiosensitizers

Extensive research within the last decade in cell culture and in rodents has revealed that phytochemicals can sensitize cervical cancer to chemoradiotherapy [89]. Phytochemicals also can sensitize tumors to different chemotherapeutic agents, which include paclitaxel, doxorubicin, 5-FU, vincristine, melphalan, butyrate, cisplatin, celecoxib, vinorelbine, gemcitabine, oxaliplatin, etoposide, sulfinosine, thalidomide, and bortezomib [89]. LY294002 and Curcumin are probably the two phytochemicals, which are widely used as chemoradiotherapy sensitizers for treatment of cervical and other cancers. Laboratory and clinical evidence has provided a strong rationale for the validation of combination of a phytochemical as a sensitizer with a therapeutic drug or radiation through clinical trials.

Chemo-sensitizers

Both drug resistance and dose-limiting toxicity limit the clinical application of a therapeutic drug for cancers [63]. To develop a novel strategy is to overcome chemoresistance and sensitize cancer cells to a drug that can enhance the therapeutic effect of the drug. Sodium butyrate (NaBT) is a histone deacetylase (HDAC) inhibitor, which has the potential to induce apoptosis of HPV-positive carcinoma cells by arresting G1 phase. Both wortmannin and LY294002 significantly enhance NaBT-mediated apoptosis [91]. Either wortmannin or LY294002, combined with NaBT, enhances the activation of caspase 3 and caspase 9 and subsequent cleavage of poly (ADP-ribose) polymerase (PARP) and also increases dephosphorylation of Rb [91]. In an *in vitro* study, cisplatin at CPI (50) targets both apoptosis and survival pathways by activating the caspase-cascade and inhibiting AKT, mTOR, p70S6K, and 4EBP1. As a chemo-sensitizer, LY294002 results in either synergistic or antagonistic effect of cisplatin [68]. Curcumin sensitizes cervical cancer cells to the therapeutic effect of Taxol. A combination of 5 nm Taxol with 5 μM curcumin augments anticancer effects more efficiently than Taxol alone by increased cytotoxicity and reduced DNA synthesis in HeLa cells [62]. The combination at the cellular level augments activation of caspases and cytochrome c release by down-regulating taxol-induced activation of NF-κB and phosphorylation of AKT [62]. Taxol in combination with curcumin may provide a superior therapeutic index and advantage in the clinic for the treatment of refractory tumors [62]. This synergistic effect was not observed in normal cervical cells, 293 cells in which Taxol down-regulates NF-κB, or HeLa cells transfected with IκBα DM. Evaluation of signaling pathways common to Taxol and curcumin reveals that this synergism is in part related to down-regulation of NF-κB and serine/threonine kinase AKT pathways by curcumin. CWE treatment activates the mitochondrial apoptotic pathway resulting in mitochondrial membrane potential loss and caspases 9 activation [80]. Curcumin enhances paclitaxel-induced cytotoxicity *in vitro* through downregulation of NF-κB and AKT pathways in cervical cancer cells [63]. This synergism exists in two mouse models *in vivo*: 1) mouse cervical multistage squamous cell carcinoma model using 3-methylcholanthrene (3-MC); and 2) a xenograft model of human cervical cancer in nonobese diabetic severe combined immunodeficient (NOD-SCID) mice using HeLa cells. Combined treatment of curcumin and paclitaxel induces a synergestic reduction in the tumor incidence and tumor size of animals compared with the individual treatments of paclitaxel or curcumin [63]. Furthermore, pre-exposure of carcinoma cells isolated from 3-MC-induced tumors to curcumin potentiates paclitaxel-induced apoptosis. Curcumin augments the antitumor action of paclitaxel by downregulating the activation and downstream signaling of antiapoptotic factors and survival signals such as NF-κB, AKT and MAPK that have significant roles in proliferation, survival, angiogenesis and metastasis [63]. Curcumin can sensitize paclitaxel to induce apoptosis of cervical cancer cells through down-regulation of NF-κB and AKT [92]. As a potent chemosensitizer, curcumin can effectively down-regulate all these survival signals induced by paclitaxel to improve the therapeutic index of paclitaxel [92].

Radio-sensitizers

Three phytochemicals LY294002, curcumin and genistein have been used as the radio-sensitizers through the PI3K/AKT pathway for treatment of cervical and other cancers in both *in vitro* experiments and preclinical trials. LY294002 can significantly radiosensitize human cervical cancer cells [6]. LY294002 significantly radiosensitized HeLa and CaSki cells with DMFs (1 log cell kill) of 1.95 and 1.37, respectively, although it alone only slightly decreased cell survival [6]. The central mechanism, by which LY294002 radiosensitizes, is *via* DNA-PK inhibition, which induces DNA double-strand break repair inhibition [93]. DNA double-strand breaks are typically repaired within 2-6 h following radiation. DNA-PK activity was significantly inhibited by LY294002. In an *in vivo* experiment, HeLa cells with sustained PI3K activity and AKT phosphorylation were injected subcutaneously into BALB/C nude mice to establish

tumor cell xenograft, which were randomly assigned to four treatments: control, LY294002 alone, radiation alone, or radiation combined with LY294002 [94]. LY294002 (100 mg/kg) alone could produce xenograft regrowth delay, but combination of LY294002 and radiation resulted in significant and synergistic suppression of tumor regrowth. LY294002 synergistically enhanced radiation efficacy *via* preventing dephosphorylation of AKT and PI3K [94]. Curcumin is a widely studied chemopreventive agent that does not radiosensitize normal human diploid fibroblasts and has a low toxicity profile in three human clinical trials [69]. Curcumin can sensitize cervical carcinoma to gamma radiation by protecting normal organs such as liver, kidney, oral mucosa, and heart from chemotherapy and radiotherapy-induced toxicity [89]. In *in vitro* experiments using HeLa and SiHa cells, curcumin scarcely affects ionizing radiation(IR)-induced activation of AKT and NF-κB, but causes a significant increase in the production of reactive oxygen species (ROS), which further led to sustained ERK1/2 activation through a ROS-dependent mechanism for curcumin radiosensitivity [69]. Curcumin can be applied either systemically or topically as an effective radiation modifying modality in the treatment of cervical cancer [69]. The preclinical studies are expected to lead to clinical trials to prove the potential of this age-old golden spice for treating cancer patients [89]. Genistein is another phytochemical that has been tested as a radiosensitizer for CaSki and ME180 cells [28]. The two cell types were more radio-sensitive at 20 and 40 μM of genistein. At 40 μM, less than 5% of Me180 cells can survive the radiation at 200-800 cGy and CaSki cells at 500 and 800 cGy. Furthermore, genistein significantly inhibits expression of Mcl-1 and causes G(2)M arrest accompanied with increase in radiosensitivity in Me180 cells, but prevents the activation of $pAKT^{(Thr\ 308)}$ to enhance radiosensitivity of the CaSki cells [28]. In a word, the three radio-sensitizers can lead to clinical trials for treatment of cervical and other cancers.

CONCLUSION

In conclusion, cervical cancer currently remains a major health problem in women worldwide because of no therapeutic HPV vaccines available. In the last decade, there has been great progress in understanding of that PI3K/AKT/mTOR pathway plays a crucial role in cervical carcinogenesis by mediating multiple cellular functions including cell growth and proliferation, metabolism, motility, migration, invasion and angiogenesis. The clinical evidence has shown that the concurrent chemoradiotherapy for advanced cervical cancer dramatically improves the local control of this disease and overall survival by triggering tumor cell apoptotic pathways involving the PI3K/AKT/mTOR signallings. The PI3K/AKT/mTOR pathway is recognized as the best target for novel cervical cancer therapy. Several phytochemicals have been reported to be strong PI3K cascade inhibitors, which are potential therapeutic agents or novel chemoradiotherapy sensitizers. Thus, it is very important to clinically identify the cervical cancer patients most likely to benefit from the reported PI3K inhibitors and combinations of PI3K inhibitors with chemoradiotherapy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

As2O3	=	Arsenic trioxide
BCDD	=	butyl 2-cyano-3, 11-dioxours-1,12-dien-24-oate
CA	=	clonogenic assays
CDK	=	cyclin-dependent kinase
CIN	=	cervical intraepithelial neoplasia
COX-2	=	cyclooxygenase-2
CRE	=	cyclic adenosine monophosphate response element
CREB	=	cyclic adenosine monophosphate response element binding protein
CXCR-4	=	C-X-C chemokine receptor type 4
CW	=	Curcuma wenyujin
DIM	=	3,3-diindolylmethane
DPPT	=	deoxypodophyllotoxin
EGCG	=	epigallocatechin-3-gallate
EGFR	=	epithelial growth factor receptor
EMT	=	epithelial-mesenchymal transition
ERK-1/2	=	extracellular signal-regulated kinase-1/2
FAK	=	focal adhesion kinase
FTS	=	Fused Toes Homolog
GRB2	=	growth factor receptor-bound protein 2
GSK3β	=	glycogen synthase kinase-3 beta
HCC	=	human cervical carcinoma tissues
HDAC	=	histone deacetylase
HFKs	=	human foreskin keratinocytes
HIF-1α	=	hypoxia-inducible factor-1alpha
HMECs	=	human mammary epithelial cells
HPV	=	human papillomavirus
HSIL	=	high grade cervical squamous intra-epithelial lesion
IGF-1	=	Insulin-like growth factor 1

IκBα DM = inhibitor kappaBalpha double mutant

JNK = c-Jun N-terminal kinase

JOT01006 = Ethyl 2- [N-m-chlorobenzyl- (2'-methyl)] anilino-4-oxo-4,5-dihydrofuran-3-carboxylate

JOTO1007 = Ethyl 2-[N-p-chlorobenzyl-(2'-methyl)] anilino-4-oxo-4,5-dihydrofuran -3-carboxylate

p-c-jun = phosphorylated-c-jun

LACC = locally advanced cervical cancer

LPA = lysophosphatidic acid

MAPK = mitogen-activated protein kinase

MEKK3 = MAP kinase kinase 3

MKK-4 = mitogen-activated protein kinase kinase-4

MKK7 = mitogen-activated protein kinase kinase 7

MAGUK = membrane-associated guanylate kinase

MMP-2 = metalloproteinase-2

MT1-MMP = membrane type 1 matrix metallo-proteinase

mTOR = mammalian target of rapamycin

c-iNOS = inducible nitric oxide synthases

N101-2 = diethyl 5,7,4'-trihydroxy flavanone N-phenyl hydrazone

NaBT = Sodium butyrate

NF-κB = nuclear factor-kappaB

NHERF-1 = Na(+)/H(+) exchange regulatory factor 1

PD184352 = 2-(2-chloro-4-iodo-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide

PD98059 = 2'-amino-3'-methoxyflavone

PDZ = post synaptic density protein (PSD95) Drosophila disc large tumor suppressor (Dlg1) and zonula occludens-1 protein (zo-1)

PI3K = phosphatidylinositol 3-kinases

PTEN = Phosphatase and tensin homolog

PPARγ = peroxisome proliferator-activated receptor γ

Rb = retinoblastoma

RhoA = Ras homolog gene family, member A

ROCK-1 = Rho-associated, coiled-coil containing protein kinase 1

ROS = reactive oxygen species

SAPK = stress-activated protein kinase

SCC = Squamous cell carcinoma

SDF-1 = Stromal Derived Factor-1

SEK-1 = extracellular signal-regulated kinase kinase 1

Skp2 = S phase kinase-associated protein 2

SOS-1 = son of sevenless homolog 1

TACC3 = transforming acidic coiled coil-3

U0126 = 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophynylthio) butadiene

TKI = tyrosine kinase inhibitor

TPD = triterpenediol

TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand

VEGF = vascular endothelial growth factor

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