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Inhibition of ErbB receptors, Hedgehog and NF-kappaB signaling by polyphenols in cancer

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1. ABSTRACT

Carcinogenesis is a multi-step process triggered by cumulative genetic alterations, which drive the progressive transformation of a normal cell into a cancer cell. Among the signal transduction pathways whose cross-talk plays an important role in neoplastic transformation are those mediated by ErbB receptors, NF-kappaB and the Hedgehog (HH)/glioma-associated oncogene (GLI) cascade. Polyphenols can be employed to inhibit the growth of cancer cells due to their ability to modulate the activity of multiple targets involved in carcinogenesis through simultaneous direct interaction or modulation of gene expression. This review will describe the cross-talk between ErbB receptors, NF-kappaB and the Hedgehog (HH)/glioma-associated oncogene (GLI) signaling pathways and the potential role of polyphenols in inhibiting this dialogue and the growth of cancer cells.

2. INTRODUCTION

Carcinogenesis is a multi-step process triggered by cumulative genetic alterations, which drive the progressive transformation of a normal cell into a cancer cell. Malignant transformation is driven by overexpression or hyperactivation of genes which support cell survival and proliferation (oncogenes) or, *viceversa*, by loss of expression or functional inactivation of genes which negatively affect cell proliferation or are able to induce cell death (tumor suppressor genes). As a result, while growth and survival of normal cells are regulated by exogenous stimuli, cells bearing oncogenes and tumor suppressor genes mutations can grow in the absence of exogenous signals and become unresponsive to negative regulators of growth and survival (1-4). Accordingly, overexpression of growth factors and/or their receptors can cause constant activation of downstream signaling pathways leading to

cell growth and survival (1-4). Besides, growth factor receptors and the downstream signal transduction effectors or transcription factors can be directly activated by mutational events (1,2). In addition, different signal transduction pathways involved in neoplastic transformation often interact with each other and potentiate oncogenic signals (2). Among the signal transduction pathways whose cross-talk plays an important role in neoplastic transformation are those mediated by ErbB receptors, NF-κB and the Hedgehog (HH)/glioma-associated oncogene (GLI) cascade. Polyphenols can be employed to inhibit the growth of cancer cells due to their ability to modulate the activity of multiple targets involved in carcinogenesis through simultaneous direct interaction or modulation of gene expression (5-7). This review will describe the cross-talk between ErbB receptors, NF-κB and the Hedgehog (HH)/glioma-associated oncogene (GLI) signaling pathways and the potential role of polyphenols in inhibiting this dialogue and the growth of cancer cells.

3. ERBB RECEPTORS SIGNALING PATHWAY

Epidermal growth factor receptor (EGFR) family members including EGFR, ErbB2 (Neu, HER2), ErbB3, and ErbB4 play a critical role in cancer development (8). Signal transduction by ErbB family receptors involves an array of ten possible homodimeric and heterodimeric combinations diversifying biologic responses to ligands of the EGF and the neuregulin (NRG) family (9). Upon ligand binding, ErbB receptors undergo dimerization and receptor trans-phosphorylation. ErbB3 lacks of intrinsic kinase activity, while ErbB2 appears to have no direct ligand (8). These events lead to the activation of the receptor tyrosine kinase which then activates by phosphorylation the downstream effector enzymes phospholipase C gamma (PLCγ), phosphatidylinositol 3-kinase (PI3K) and Src, and binds the adaptor proteins Grb2 and Shc, which contain multiple protein interaction motifs able to recruit and activate the RAS GTPase (8). PLCγ induces the formation of diacylglycerol, which triggers the serine-threonine kinase protein kinase C (PKC), which in turn activates various transcription factors. PI3K activates the kinase Akt (protein kinase B), which is involved in cell proliferation and inhibition of apoptosis (10). RAS proteins activate RAF kinases which initiate a phosphorylation cascade, which activates the classical mitogen-activated protein kinases (MAPKs) signaling pathway up to the extracellular signal regulated kinases 1 and 2 (ERK1/2) which translocate to the nucleus and activate several transcription factors, including activator protein-1 (AP-1), c-Myc, etc. (10). The MAPK/ERK pathway represents one of the major signaling cascades regulating cell proliferation. Other terminal serine/threonine kinases include c-Jun amino-terminal kinases (JNK1/2/3), p38 kinases and ERK which are activated by stress and growth factors (10). JNK and p38 kinases mainly regulate cell differentiation and apoptosis (10). Given the established role of abnormal ErbB receptors activation in tumor cell proliferation, survival and metastasis, many efforts are currently made to identify drugs that inhibit their signaling pathway (11-16). In addition, ErbB2 oncogenic potential combined with the high level overexpression in tumor tissue and cell surface localization render this receptor a suitable target for immunotherapeutic approaches (17-28). The principal ErbB receptors-mediated signaling pathways are illustrated in Figure 1, panel A.

4. NF-κB SIGNALING PATHWAY

The persistent activation of NF-κB has been linked with tumor development. Indeed, this transcription factor can support cell transformation, tumor cell survival, proliferation, invasion, metastatization and neoangiogenesis (29). NF-κB is a homodimeric or heterodimeric transcription factor which can be formed by different combinations of five subunits (RelA/p65, c-Rel, RelB, NF-κB1/p50, and NF-κB2/p52). This transcription factor is ordinarily sequestered in an inactive state in the cytoplasm by the inhibitors of NF-κBs (IκBs). Activation of the IκB kinase (IKK) complex, which includes two catalytic (IKKα and IKKβ) and one regulatory (IKKγ/NEMO) subunit, leads to phosphorylation of the IκBs, targeting them for ubiquitination and proteasomal degradation. The degradation of IκBs permits NF-κB to translocate to the nucleus, where it activates different genes involved in inflammation, cell growth, cell survival and invasivity (30). Indeed, the activation of NF-κB represents a critical link between inflammation and tumorigenesis and is triggered by growth factors, cytokines and many stress stimuli (29-31). The constant activation of NF-κB in cancer cells is supported by the high levels of inflammatory mediators (tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, prostaglandin E2 (PGE2) and reactive oxygen species) within the tumor microenvironment (24,29-32).

Cyclooxygenases (COX-1 and COX-2), are key enzymes responsible for the biosynthesis of prostaglandins (PGs) and thromboxanes (TXs) from arachidonic acid. These enzymes and their products play a key role in inflammation and tumor progression (33). COX-2 expression is highly induced by growth factors, proinflammatory cytokines and other tumor promoters and its activity is dysregulated in several types of cancer (33). In addition, COX-2-derived PGE2 is the principal prostaglandin found in human tumors and it promotes tumor progression by inducing cell proliferation, migration, invasion, angiogenesis and by inhibiting apoptosis (33). Conversely, COX-1 is constitutively expressed (33). COX-2 expression is mainly controlled by NF-κB, AP-1 and their upstream kinases, MAPKs (33). In addition, to attain its full biological activity, NF-κB must undergo different post-translational modifications, including acetylation. Acetylation at different lysine residues in the RelA/p65 subunit affects definite functions of NF-κB, including transcriptional activation, DNA binding, and assembly with its inhibitor IκB (34,35). NF-κB acetylation is accomplished by different histone acetyltransferases (HATs) (34,35). NF-κB canonical signaling pathway is illustrated in Figure 1, panel B.

5. THE HEDGEHOG (HH)/GLIOMA-ASSOCIATED ONCOGENE (GLI) SIGNALING PATHWAY

The Hedgehog (HH)/glioma-associated oncogene (GLI) cascade is a complex signaling pathway

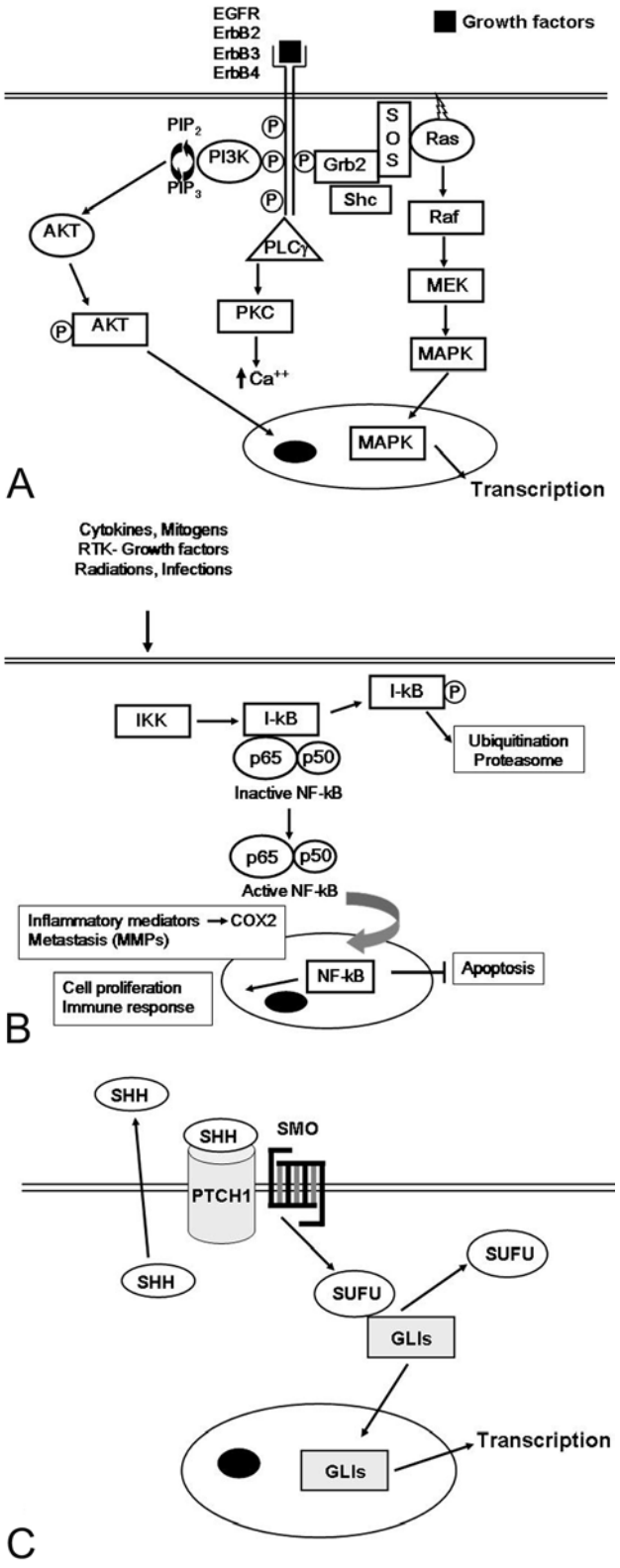

Figure 1. ErbB receptors, NF-κB and Hedgehog/GLI signaling pathways. Panel A: ErbB receptors mediated-signaling. Panel B: the "canonical" signaling pathway which leads to NF-κB activation. Panel C: Hedgehog (HH)/glioma-associated oncogene (GLI) mediated-signaling.

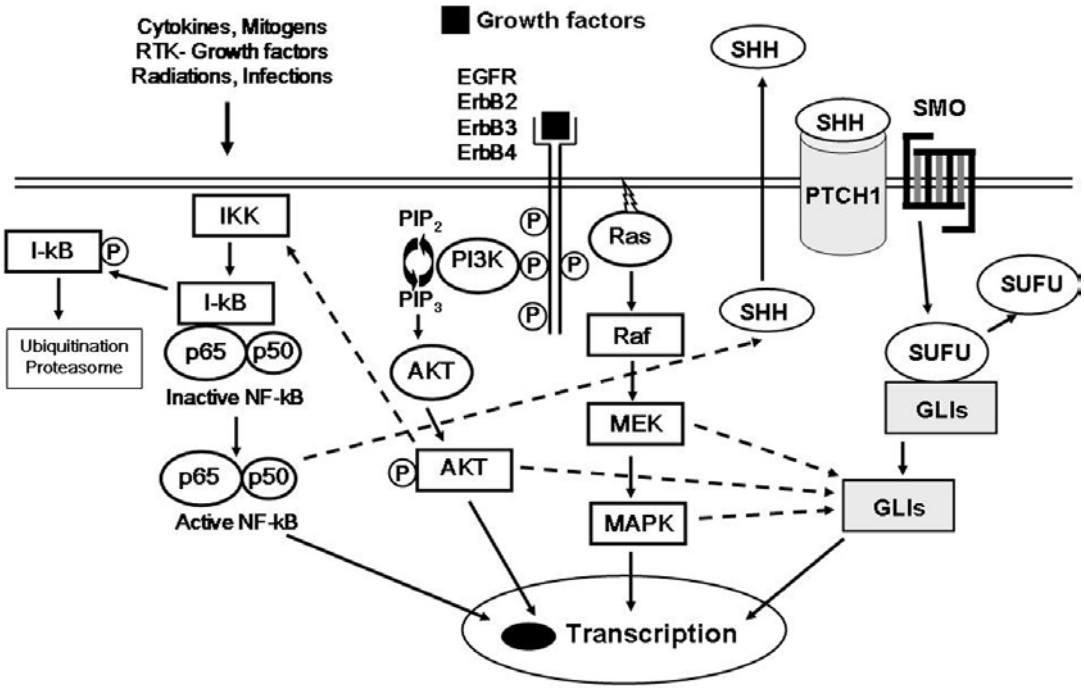

Figure 2. Cross-talk between ErbB receptors, Hedgehog/GLI and NF-κB signaling pathways in cancer cells. Cross-talk between signaling pathways is shown with a dashed line.

which plays a crucial role in vertebrate embryogenesis by controlling cell fate, proliferation, survival and differentiation (36-38). Three HH homologues have been identified in vertebrates: Sonic Hedgehog (SHH), Desert Hedgehog (DHH) and Indian Hedgehog (IHH). These three ligands initiate HH signaling through the binding to transmembrane protein receptors named Patched (PTCH 1/2) which are located at the base of a non-motile structure protruding from the cell surface called "primary cilium" (36-38). While in the absence of the HH ligand, PTCH1 receptor represses signal transduction by inhibiting the transmembrane protein Smoothened (SMO) to enter the cilium, upon ligand binding the inhibitory function of PTCH1 receptor on SMO is abolished, resulting in SMO activation. SMO enters the cilium and promotes the activation of cytoplasmic GLIs and their translocation to the nucleus, where they initiate transcription of HH target gene products (36,37). Three GLI proteins are involved in this signaling: GLI1 and GLI2 have activating effects, while GLI3 antagonizes the function of SHH–GLI1 (36-38). Among the negative modulators of HH signaling pathway, the suppressor of fused (SUFU) protein can bind and maintain GLI proteins in the cytoplasm in the absence of HH ligands thus preventing their nuclear translocation (37). The HH signaling has an important role along with other signaling pathways such as those mediated by ErbB receptors (8,9) and Wnt/β-catenin (39) in regulating embryonic development, differentiation and cell survival (36).

Aberrant HH signaling, which can be achieved by mutational inactivation of PTCH, aberrant expression of its ligand, constitutive activation of SMO or gene amplification of the GLI transcription factors, has been implicated in initiation and/or maintenance of different cancer types including basal cell carcinoma (BCC), gastrointestinal, lung, brain tumors and rhabdomyosarcoma (36-38). In addition, there is evidence that dysregulation of HH signaling can be involved in development and progression of breast cancer (38,40). Indeed, the expression of SHH was found to be upregulated in early stage breast carcinoma, indicating that the upregulation of SHH may be an early event in breast carcinogenesis (41). HH signaling can be blocked at many levels by a variety of small molecules that target different members of its pathway. To date, several antagonists of SMO have entered clinical trials (42). A schematic representation of the HH-GLI signaling pathway is illustrated in Figure 1, panel C.

6. CROSS-TALK BETWEEN ERBB RECEPTORS, HH/GLI AND NF-κB SIGNALING PATHWAYS

Accumulating evidence indicate that a complex interplay can occur between ErbB receptors, HH signaling and NF-κB pathway, and between HH and NF-κB signaling (Figure 2). It was reported that EGFR signaling via RAF/MEK/ERK modulates the target gene expression profile of GLIs in epidermal cells (43) and that SHH induces EGF-dependent matrix infiltration in HaCaT keratinocytes and that constitutive SHH expression is associated with increased phosphorylation of EGFR (44). Cooperation of EGFR signaling with HH/GLI was demonstrated to promote cancer cells transformation and proliferation. Eberl *et al.* identified a group of HH-EGFR cooperation response genes (i.e. JUN, SOX9, SOX2, FGF19, CXCR4), whose expression was directly regulated by GLI, but synergistically increased by EGFR signaling.

These genes were important for determining the oncogenic phenotype of both BCC and tumor-initiating pancreatic cancer cells (45).

The PI3K/Akt and MAP kinase cascade (36,37) are at the crossroads of the cooperative EGFR/ErbB2 receptors-HH/GLI signaling pathways. Indeed, it was provided evidence that endogenous RAS-MEK and Akt signaling pathways regulate nuclear localization and transcriptional activity of GLI1 in melanoma and other cancer cells (46). Riobò *et al.* demonstrated that PI3K and Akt activities are crucial for GLI-dependent SHH signaling. In addition, they provided evidence that stimulation of PI3K/Akt by IGF-I potentiates GLI transcriptional activity in the presence of low amounts of SHH (47). The same authors identified PKC-delta and MEK-1 as essential, positive regulators of GLI-mediated HH signaling (48). Schnidar *et al.* reported that EGFR signaling synergizes with GLI1 and GLI2 to selectively activate transcription of a subset of target genes via stimulation of RAS/RAF/MEK/ERK signaling; this induces JUN/activator protein 1 activation, which is crucial for oncogenic transformation. Further, these authors reported that combined inhibition of EGFR/MEK/ERK/JUN and HH/GLI signaling efficiently reduces growth of BCC (49). In addition, Seto *et al.* reported that PTCH expression was correlated with ERK1/2 phosphorylation and SHH expression in gastric cancers and that MAPK signaling regulates GLI activity via a SUFU-independent process (50). In agreement with the involvement of both HH and ErbB signaling in proliferation of androgen-independent prostate cancer cells, a synergistic effect of HH and ErbB inhibitors on prostate cancer cell growth was observed (51). In addition, the combined treatment with docetaxel and EGFR (gefitinib) and HH signaling (cyclopamine) inhibitors induced a higher rate of apoptotic death of prostate cancer cells compared with that obtained with individual agents (36).

A further level of cross-talk between ErbB receptors and HH signaling is likely to involve NF-κB. Hinohara *et al.* reported that heregulin, a ligand for ErbB3, induced mammosphere formation through a PI3K/NF-κB pathway in human breast cancer (52). It was also reported that IKKα plays an important role in controlling the ability of ErbB2 to activate NF-κB through the canonical pathway and that IKKα controls invasion of ErbB2 positive breast cancer cells (53). In addition, transactivation of ErbB2 provoked by TNF-α induced NF-κB activation and breast cancer cell proliferation (54). The activation of NF-κB is also capable of increasing ErbB2 activity. Indeed, it was shown that NF-κB activity enhances ErbB2-mediated mammary tumorigenesis *in vivo* by promoting both growth and survival via the stimulation of tumor vasculogenesis (55), and that IKKα has an important role on ErbB2-induced oncogenesis, providing signals that maintain mammary tumor-initiating cells (56). Moreover, activation of NF-κB in human breast cancer is confined predominately to the estrogen receptor-negative subtype of cancers, particularly those that express EGFR and ErbB2 receptors (57). Indeed, ErbB2 activates NF-κB, and EGFR/ErbB2 overexpression participates or enhances the aberrant activity of NF-κB in cancer cells (53,58). It was earlier reported that ErbB2 activates NF-κB via a PI3K to Akt signaling pathway (59). Makino *et al.* reported that upregulation of IKKα and IKKβ by the integrin-linked kinase/Akt pathway is required for the ErbB2-mediated NF-κB anti-apoptotic pathway (60). Likewise, it was demonstrated that ErbB2 constitutively activates the Akt/NF-κB anti-apoptotic cascade to confer resistance to TNF on cancer cells (61).

A complex interplay arises also between PGE2 and growth factors mediated-signaling. Indeed, PGE2 can transactivate EGFR. In addition, it can stimulate the production of angiogenic growth factors, such as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF), which in turn increase COX-2 expression. Signaling by PGE2 receptors further involves the activation of PI3K/Akt pathway and of the RAS/MAPK pathway, which is also able to enhance COX-2 expression, and upregulates the transcriptional activity of NF-κB, whose target genes include COX-2, whose expression is also controlled by AP-1 and their upstream kinases, MAPKs (29,33,62).

As for the interplay between NF-κB and HH signaling, it has been demonstrated that NF-κB can directly regulate SHH expression *in vitro* and *in vivo,* and promote pancreatic cancer cell proliferation and apoptosis resistance via the SHH pathway (63,64).

7. POLYPHENOLS

Polyphenols are natural compounds expressed in many medicinal plants. They exert various biological functions in plants, such as modulation of activity of enzymes and cell receptors, and show beneficial effects on human health including anti-inflammatory, anti-microbial, anti-cancer and immunomodulatory activities (6). They are classified in two large groups, flavonoids and non-flavonoids, based on the number of phenol rings and structural elements bound to these rings.

Flavonoids are the most abundant polyphenols compounds present in edible plants and in our diet, and can be divided in several classes according to different functional groups and level of oxidation in the C-ring. The most important classes are flavonols, flavones, flavan-3-ols, anthocyanins, flavanones and isoflavones. Among non-flavonoids, the most important classes frequently found in foods are phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), stilbenes and lignans (65-67).

8. POLYPHENOLS AND ERBB RECEPTORS SIGNALING PATHWAY IN CANCER CELLS

Among the different mechanisms explaining the anti-cancer effects of polyphenols, the modulation of ErbB receptors signaling cascades is one of the most important. Several studies have shown that a variety of polyphenols exhibits the ability to inhibit EGFR, ErbB2/*neu* and ErbB3 pathways both *in vitro* and *in vivo*.

In this regard, many investigations have demonstrated that the green tea polyphenol

epigallocatechin-3 gallate (EGCG) is one of the most promising natural compounds that can be used to inhibit ErbB receptors downstream signaling in several types of tumors. Pianetti *et al*. reported that EGCG was able to counteract the growth of ErbB2/*neu*-overexpressing mouse mammary tumor NF639 and SMF cell lines. EGCG reduced the basal phosphorylation and constitutive activation of ErbB2/*neu* and inhibited the PI3K, Akt kinase and NF-κB signaling pathway (68). In addition, a model for demonstrating the anti-tumor activity of EGCG at multiple steps in ErbB2- or/and ErbB3-overexpressing breast cancer cells has been proposed by Pan *et al.* The authors suggested that EGCG might interfere with heterodimerization and tyrosine phosphorylation of ErbB2-ErbB3 because it competes with Heregulin-β1, an ErbB3 ligand (69). Masuda *et al.* examined the anti-tumor activity of EGCG on human head and neck squamous cell carcinoma (HNSCC) and human breast cancer cell lines in which EGFR was constitutively activated. EGCG inhibited the activity of EGFR and blocked the activity of Stat3 and Akt in both cell lines (70). The same authors also examined the anti-tumor activity of EGCG on HNSCC and human breast cancer cell lines in which ErbB2 was constitutively activated. They reported that EGCG inhibited phosphorylation of ErbB2 in both cell lines, determining the inhibition of Stat3, c-*fos* and cyclin D1 promoter activity, and a reduction of cyclin D1 and Bcl-x_L protein levels (71). EGCG potentiated the anti-metastatic effects of gefitinib, a tyrosine kinase inhibitor, on CAL-27 human oral squamous carcinoma cells. Combined treatment with EGCG and gefitinib enhanced the suppression of phosphorylation of EGFR and suppressed the phosphorylation of ERK, JNK, p38 and Akt (72). EGCG was also able to arrest growth and revert the transformed phenotype of ErbB2-overexpressing human breast cancer BT-474 cells resistant to trastuzumab, a humanized monoclonal antibody used for immunotherapy of ErbB2-overexpressing tumors. Resistance to trastuzumab is due to activation of Akt signaling cascade and loss of CDK inhibitor $p27^{Kip1}$ expression. Inhibition of proliferation of these cells by EGCG was mediated by its capacity to reduce Akt activity that determines an increase of FOXO-3a and $p27^{Kip1}$ levels. In agreement with these results EGCG reverted resistance to trastuzumab (73). In another study, the ErbB2/*neu*-transformed breast cancer cell line NF639 resistant to EGCG has been isolated. These cells showed enhanced activation of Akt and NF-κB, elevated MAPK signaling and a reduction of tyrosine phosphorylation of the ErbB2/*neu* receptor, that were responsible for invasive phenotype. Surprisingly, the authors observed that the treatment with EGCG in combination with dexamethasone, a potent inhibitor of NF-κB, or in combination with U0216, a MAPK inhibitor, blocked the growth and reverted the invasive phenotype of NF639 cells, suggesting that combinatorial treatments were more efficient than treatment with EGCG alone to enhance the potency of therapy against ErbB2/*neu*-overexpressing tumors (74). EGCG showed similar anti-tumor effects also in human esophageal, colon cancer and non-small cell lung cancer (NSCLC) cells. Hou *et al.* observed that EGCG inhibited the phosphorylation of EGFR in esophageal squamous carcinoma KYSE150 cells and in epidermoid squamous carcinoma A431 cells (75). EGCG was able to decrease the phosphorylation of ErbB3, EGFR and ErbB2, thus inhibiting their downstream pathways, and to reduce the cellular levels of these proteins in the colon carcinoma cell line SW837, that displayed high levels and a constitutive activation of ErbB3 (76). Adachi *et al*. showed that EGCG inhibits the activation of EGFR and causes its internalization or degradation by altering plasma membrane organization in human colon cancer cells (SW480) (77,78). They also demonstrated that EGCG (50 μM) caused phosphorylation of EGFR at $Ser^{1046/1047}$, a site that is critical for its down-regulation, through activation of p38 kinase (79). Milligan *et al.* reported that EGCG in combination with erlotinib, an antagonist of EGFR, inhibited growth of erlotinib-sensitive and erlotinib-resistant NSCLC cell lines more strongly than either agent alone, blocking EGFR phosphorylation and affecting EGFR downstream pathways. In particular, EGCG inhibited phosphorylation of Akt and ERK1/2 (80). Similarly, genistein enhanced the inhibitory effects of erlotinib and gefitinib on EGFR phosphorylation in NSCLC cell lines, causing the suppression of EGFR and Akt expression as well as NF-κB inactivation (81).

A study by Fridrich and co-workers investigated the inhibitory properties of EGCG, the anthocyanin (ACN) aglycon delphinidin and the flavonol quercetin on EGFR and ErbB2 receptor activities. Delphinidin was able to suppress EGFR phosphorylation in a human colon carcinoma cell line (HT29) (IC_{50}: 54±11 μM) and in a human vulva carcinoma cell line (A431) (IC_{50}: 71±32 μM). Delphinidin was also able to down-regulate the phosphorylation of ErbB2 in A431 cells (IC_{50}= 60±21 μM) and to inhibit the activity of the downstream targets ERK1/2. In addition, the flavonol quercetin was more effective than delphinidin in inhibiting EGFR phosphorylation (IC_{50}: 0.6±0.1 μM) and in down-regulating ERK1/2 activities in HT29 cells. Conversely in A431 cells, quercetin had a marginal effect in inhibiting the activity of ErbB2 receptor (IC_{50}: ≥150 μM). Finally, in A431 cells EGCG exhibited significant inhibitory properties on the phosphorylation of ErbB2 only at high concentrations (IC_{50}: ≥150 μM). The authors concluded that quercetin is most effective against the EGFR, whereas delphinidin exhibits preference towards the ErbB2 receptor (82). The anti-cancer properties of delphinidin, and in particular its effect on EGFR signaling pathway, have been studied by Afaq *et al.* Delphinidin (5-40 μM) inhibited the phosphorylation of EGFR and of its downstream targets Akt, ERK1/2, JNK1/2/p38 in EGFR-positive AU-565 and immortalized MCF-10A breast cancer cells in a concentration-dependent manner. Delphinidin was also able to inhibit EGF-induced auto-phosphorylation of EGFR in the same cell lines (83). Another report by Teller *et al.,* examined the ability of delphinidin in inhibiting the kinase activity of EGFR, ErbB2, VEGFR-2/3 and IGF-1R. Delphinidin was able to inhibit the protein tyrosine kinase activity of all receptors at concentrations ≥5 μM in a cell-free test system. Delphinidin was able to decrease the ligand-induced phosphorylation of EGFR and ErbB2 receptors in A431 cells (IC_{50}:72±32 μM for EGFR, IC_{50}: 51±23 μM for ErbB2) (84). In a recent report by Ozbay *et*

al., the authors employed delphinidin to determine the *in vitro* properties of this ACN on the growth of seven established breast cancer cell lines of varying molecular subtypes including ErbB2-overexpressing, ER-positive, and triple negative cells. Delphinidin (12.5-100 μg/mL) was able to inhibit proliferation of HCC1806, MDA-MB-231, MDA-MB-468, SKBR3, MDA-MB-453, BT-474 and MCF-7 breast cancer cells. In particular, delphinidin induced the highest level of apoptosis (4- to 6-fold) in the ErbB2-overexpressing lines SKBR3 and BT-474 as compared to (2-fold) the triple negative lines HCC1806 and MDA-MB-231. In addition, delphinidin suppressed ErbB2 signaling in SKBR3 cells, which overexpress ErbB2, through the down-regulation of the phosphorylation of ErbB2 and the downstream targets ERK1/2 and Akt. Inhibition of ERK1/2 by delphinidin was also observed in HCC1806 and MDA-MB-468 cells. Thus, the authors suggested that delphinidin is an inhibitor of ErbB2/MAPK signaling (85).

A study by Xu and co-workers investigated the effect of the ACN cyanidin-3-glucoside (C3G) on ethanol-mediated migration/invasion of breast cancer cells expressing high levels of ErbB2. MCF-7 cells overexpressing ErbB2 (MCF-7^{ErbB2}), MDA-MB-231 and BT-474 breast cancer cells were treated with ethanol, in presence or absence of C3G for 48h. It was found that ethanol increased migration/invasion of breast cancer cells, but the treatment with C3G (10-40 μM) was able to inhibit ethanol effects in a dose-dependent manner in MCF-7^{ErbB2} and MDA-MB-231 cells. The effect of C3G on BT-474 breast cancer cells was not dose-dependent (86). Even flavones, such as apigenin and luteolin, displayed inhibitory activity against ErbB receptors. Masuelli *et al.* demonstrated that apigenin reduces ligand-induced phosphorylation of EGFR and ErbB2 and impairs their downstream signaling in HNSCC (12). In the ER-negative MDA-MB-231 breast cancer cells, luteolin exerted its inhibitory activity on cell proliferation through the suppression of EGFR phosphorylation, leading to the inactivation of the MAPK and PI3K/Akt pathways. In addition, a diet supplementation with luteolin determined a strong reduction of tumor volume in female athymic nude mice implanted with MDA-MB-231 cells (87).

The inhibitory properties of another plant polyphenol, tannic acid, on EGFR tyrosine kinase has been investigated in a study by Yang *et al*. The authors demonstrated that tannic acid was able to strongly inhibit tyrosine kinase activity of EGFR *in vitro* (IC_{50}= 323 nM). In addition, tannic acid inhibited the EGF-induced EGFR auto-phosphorylation in Swiss 3T3 mouse fibroblasts in a concentration-dependent manner. Tannic acid was also able to inhibit the growth of HepG2 cells previously stimulated with EGF. The authors also investigated the interaction of tannic acid with EGFR in a molecular modeling study. They suggested that tannic acid could be docked into the ATP binding pockets of EGFR, since the inhibition of EGFR kinase activity was found to be ATP-competitive (88).

Another report by Katdare *et al.* investigated the effects of soy isoflavone genistein against breast cancer. Genistein was able to inhibit proliferation of the human breast epithelial cell line 184-B5 overexpressing ErbB2/*neu* and this effect was due to the inhibition of the tyrosine kinase activity of the receptor (89). Moreover, genistein induced the suppression of BT-474 human breast cancer cells growth by affecting ErbB2 phosphorylation and protein expression (90).

The effect of oak ellagitannins on EGFR has been studied by Fridrich *et al*. in human colon carcinoma cells. The aglycones castalagin (IC_{50}: 11.1±1.8 μM) and vescalagin (IC_{50}: 22.4±2.6 μM) and the C-glycosides roburin E (IC_{50}: 16.7±1.4 μM) and grandinin (IC_{50}: 28.9±2.6 μM) inhibited the growth of HT29 colon cancer cells. Besides, these ellagitannins were able to inhibit the tyrosine kinase activity of EGFR in nanomolar concentration. Finally, grandinin (IC_{50}: 35±0.3 μM) and castalagin (IC_{50}~ 10 μM) also inhibited the auto-phosphorylation of EGFR in HT29 colon carcinoma cells (91).

The same authors explored the effect of procyanidins on EGFR in human colon carcinoma cells. In particular, they isolated from grape seed extracts the dimeric procyanidins B1 (PB1), B2 (PB2), B3 (PB3), B4 (PB4), the trimeric procyanidin C1 (PC1) and tetrameric procyanidin cinnamtannin A2 (PA2). The dimeric procyanidins were able to inhibit the protein tyrosine kinase activity of EGFR with IC_{50} values of about 10 μM while the trimeric (PC1) and tetrameric (PA2) procyanidins inhibited the activity of EGFR with IC_{50} values of 1.3±0.4 μM and 0.2± 0.06 μM, respectively. It was also found that PC1 was able to inhibit EGFR auto-phospohorylation in HT29 colon carcinoma cells with an IC_{50} value of 35±15 μM, while PA2 affected the auto-phosphorylation of EGFR only at concentrations up to 50 μM (92).

Another plant polyphenol, quercetin, has been evaluated for its anti-tumor activity in breast cancer cells. In particular, a study by Jeong *et al*., investigated the effect of quercetin in inhibiting ErbB2 signaling pathway. The authors observed that quercetin induced the ubiquitinylation and down-regulation of ErbB2/*neu* in SKBR3 breast cancer cells. Quercetin treatment (100-200 μM) induced a decrease in ErbB2/*neu* protein level and a dephosphorylation of PI3K and Akt in this cell line. They also observed that quercetin was able to inhibit the tyrosine kinase activity of ErbB2/*neu* and suggested that quercetin could bind to ErbB2/*neu* and cause an alteration in its protein structure, which could lead to its degradation (93).

In studies conducted by Menendez *et al.* it has been determined the relationship between chemical structures of polyphenols derived from extra-virgin olive oil (EVOO) and their ability to inhibit phosphorylation of ErbB2 in MCF-10A breast cancer cells engineered to overexpress the wild type form of human ErbB2, and in human breast cancer SKBR3 and MCF-7 cell lines overexpressing ErbB2. The authors observed that among EVOO-derived polyphenols, lignans and secorroids, which have a complex chemical structure, were more effective than single phenols and phenol acids in suppressing ErbB2 phosphorylation and reducing ErbB2 protein expression in

all cell lines, suggesting that the presence of more phenol rings was necessary to exert inhibitory effects on ErbB2 activity (94,95). The anti-cancer effects of EVOO-derived polyphenols were also tested on the human breast cancer JIMT-1 cell line, which overexpresses ErbB2 and has cross-resistance to multiple EGFR/ErbB2-targeted drugs, such as trastuzumab, gefitinib, erlotinib and lapatinib. Even in these cells, lignans and secorroids showed a strong ability in suppressing phosphorylation and biological activity of ErbB2 and in determining an inhibition of ErbB2 downstream signaling cascades. In particular, the treatment with EVOO-derived polyphenols resulted in a reduction of endogenous levels of phosphorylated Akt, MAPKs, Stat3 (96).

Another polyphenol that exerts a potent effect on EGFR/ErbB2/ERbB3 activity, influencing its downstream cascade signaling, is curcumin. A study by Hong *et al*. demonstrated that curcumin was able to inhibit ErbB2 tyrosine kinase activity, by depleting ErbB2 protein. Curcumin down-regulated ErbB2 protein by disrupting its binding with the molecular chaperone GRP94 in the endoplasmic reticulum (97). A recent report investigated the molecular mechanism underlying curcumin-induced ErbB2 depletion. Curcumin induced association of the ubiquitin ligase CHIP with ErbB2 and the subsequent ubiquitination of ErbB2. The kinase domain of ErbB2 was required for curcumin-induced ErbB2 ubiquitination and degradation (98). Squires *et al.* investigated the ability of curcumin to modulate EGFR-mediated signaling pathways in human breast cancer MDA-MB-468 cells and observed that curcumin inhibited EGFR phosphorylation, leading to a suppression of *c-fos* expression, and ERK activity (99). Curcumin was also shown to induce apoptosis of triple-negative breast cancer MDA-MB-231 cells, an aggressive breast cancer phenotype in which expression of the estrogen receptor (ER), progesterone receptor (PR) and ErbB2 is lacking. After treatment with curcumin the expression of phosporylated forms of EGFR and ERK1/2 was significantly decreased (100). In addition, the growth of MDA-MB-231 cells was also suppressed *in vitro* and *in vivo* using a combinatorial treatment with curcumin and EGCG. After treatment, changes in the expression of proteins involved in cell proliferation were analyzed and a significant reduction of EGFR, phospho-EGFR, Akt and phospho-Akt levels was detected. Furthermore, athymic nude female mice implanted with MDA-MB-231 cells and treated with curcumin plus EGCG showed a 49% reduction of tumor volume when compared with untreated mice (101). Sun *et al*. demonstrated that curcumin inhibited cell proliferation and induced cell cycle arrest at G1 phase (30 μM) and apoptosis (50 μM) in MDA-MB-231 cancer cells overexpressing ErbB2. It has also been observed that curcumin inhibited the phosphorylation of Akt and MAPKs. Moreover, curcumin increased expression levels of p27, by down-regulating Skp2 and ErbB2 (102). Another report by Lai *et al*. analyzed the effect of curcumin in the treatment of ErbB2-overexpressing breast cancer and its interaction with herceptin. Curcumin affected the growth of cancer cells and induced a dose-dependent down-regulation of ErbB2 oncoprotein, phospho-Akt, phospho-MAPK, and NF-κB in both BT-474 and herceptin-resistant SKBR3 cells (103). The ability of curcumin to inhibit EGFR downstream signaling was also reported in tumors affecting the gastro-intestinal tract. It has been reported that curcumin suppressed expression of ErbB2 and down-regulated expression of cyclin D1 in gastric cancer cells, determining an arrest of proliferation and invasion of these cells (104). Majumdar *et al.* described the synergistic effect of curcumin and resveratrol on proliferation of colon cancer HCT-116 cells both *in vitro* and *in vivo*. These two polyphenols resulted in the inhibition of the constitutive activation of EGFR and IGF-1R *in vitro* and in reduction of tumor growth and induction of apoptosis together with attenuation of NF-κB *in vivo,* in SCID mice transplanted with HCT-116 cells (105). Moreover, curcumin was demonstrated to enhance the anti-tumor effects of biological and chemical drugs used in the treatment of colon cancer. For example, it has been demonstrated that curcumin enhanced inhibition of growth and transformation mediated by dasatinib, an inhibitor of Src family kinases (SFK), in several types of colon cancer cells. Colon cancer cells treated with curcumin in combination with dasatinib displayed reduction of EGFR, ErbB2 and ErbB3 levels as well as a strong suppression of c-Src and IGF-1R phosphorylation. In addition, the combination therapy decreased the phosphorylated forms of Akt and ERKs and caused a strong attenuation of the DNA binding activity of NF-κB. Finally, the anti-proliferative activity of curcumin plus gefitinib has been detected *in vivo* using C57BL/6J-APCMin+/− mice which develop spontaneous intestinal adenoma. Mice treated with the combined therapy showed a significant regression of intestinal tumors (90-99%), an event associated with the induction of apoptosis by curcumin and gefitinib (106). Curcumin has also shown the ability to enhance the effects of 5-fluorouracil and oxaliplatin therapy (FOLFOX) in inhibiting growth of HCT-116 and HT29 colon cancer cells. These events were associated to the decreased expression and activation of EGFR, ErbB2, ErbB3 and IGF-1R that led to inhibition of Akt phosphorylation (107,108). Inhibitory effects on EGFR activation and expression were also exerted by curcumin in other types of tumor, such as bladder, prostate and lung tumors. Chadalapaka *et al.* demonstrated that curcumin in association with betulinic acid caused the decrease of EGFR levels and the suppression of phosphorylation of Akt in KU7 and 253JB-V human bladder cancer cells (109). Similar effects were detected in human LNCaP and C4-2B prostate cancer cells in which curcumin determined a down-regulation of EGFR and ErbB2 expression (110). In addition, human prostate cancer PC-3 cells treated with curcumin in combination with β-phenylethyl isothiocyanate showed a drastic suppression of EGFR phosphorylation that resulted in the prevention of Akt and PI3K activation and in suppression of NF-κB pathway through attenuation of IκB-α phosphorylation. The inhibition of multiple pathways associated with EGFR activity promoted the activation of apoptosis and the arrest of proliferation of these cells (111). Lev-Ari and co-workers assessed the effects of curcumin on pancreatic and lung adenocarcinoma cells. Cell lines co-expressing COX-2 and EGFR (PC-14 and p34, respectively) and cells expressing EGFR but deficient in COX-2 (H1299 and Panc-1, respectively) were treated with curcumin (0-50 μM

for 72h). Curcumin inhibited cell survival and induced apoptosis of all cell lines, but the effect was higher in PC-14 and p34 cells, which express COX-2. Moreover, in these two cell lines, curcumin demonstrated to decrease COX-2, EGFR and phospho-ERK1/2 expression in a dose dependent-manner (112). The effects of curcumin in human lung adenocarcinoma cells were also investigated by Lee *et al*. The results of this study showed that curcumin potentiates the anti-cancer effects of gefitinib *in vitro* employing CL1-5, A549 and H1975 cells and in xenograft mouse models. Curcumin exerted these effects through the inhibition of proliferation, and EGFR phosphorylation, and the induction of EGFR ubiquitination and apoptosis (113). Finally, curcumin induced apoptosis of rhabdomyosarcoma and osteosarcoma cells and strongly reduced Akt expression levels as well as phospho-ERK levels in malignant rhabdoid SJ-RH4 cells (16).

It should also be considered that several studies analyzed the direct effects of polyphenols on the MAPK signaling pathway without investigating their role on ErbB receptors, since these kinases represent the principal read out of the activation of tyrosine kinase receptors. Shin *et al*. studied the anti-proliferative activity of anthocyanins derived from *Vitis coignetiae* Pulliat on the human colon cancer cell line HCT-116. These flavonoids inhibited growth and induced apoptosis of these cells in a dose-dependent manner. Further, the authors found that apoptosis was associated with the activation of p38 kinase and the inactivation of Akt (114). Ho *et al*. investigated the effects of the anthocyanin peonidin 3-glucoside (P3G) in lung cancer cells. They found that P3G inhibited the invasion, motility and secretion of MMP-2, MMP-9, and urokinase-type plasminogen activator (u-PA) of these cells. These effects were partly due to the decrease of ERK1/2 phosphorylation and the inactivation of AP-1 (115).

Gopalakrishnan *et al*. focused their research in assessing the modulation of AP-1 protein and MAPK pathway by flavonoids. In particular, they found that quercetin, chrysin, genistein and kaempferol were able to induce AP-1 in human prostate cancer cells (PC-3). In addition, they demonstrated that kaempferol, apigenin, genistein and naringenin induced the phosphorylation of JNK and ERK in the same cells. They also demonstrated that the JNK pathway is involved in the induction of AP-1 by genistein, while the MEK pathway is involved in the induction of AP-1 by kaempferol (116).

The anti-cancer effect of apigenin (5-40 μM) on androgen-responsive human prostate cancer LNCaP cells and androgen-refractory PC-3 cells was also investigated. Apigenin inhibited cell growth, arrested cell cycle in G0/G1 phase and decreased the phosphorylation of Rb protein in these cell lines. However, although apigenin increased phosphorylation of ERK1/2 and JNK1/2, it reduced phosphorylation of ELK-1 and *c-fos* protein expression. In addition, apigenin reduced expression of cyclin D1 as well as expression and phosphorylation of p38 kinase and PI3K/Akt (117). In another study evaluating the anti-cancer effects of apigenin on anti-estrogen-sensitive and -resistant breast cancer cells, a biphasic effect of apigenin was observed. In fact, at low concentrations, apigenin induced cell growth by activating ERα-mediated gene expression, while at high doses it inhibited cell growth through reduction of ERα protein levels and inhibition of the activities of multiple kinases involved in anti-estrogen resistance (p38, MAPK, PKA and PI3K/Akt) (118).

The effects of flavanone and 2'-OH flavanone on MAPK signaling have been investigated by Hsiao *et al*. in lung cancer cells (A549). These compounds (10-50 μM) inhibited the phosphorylation of ERK1/2 and p38 kinase and the activation of NF-κB and AP-1, leading to a decreased expression of MMP-2 and u-PA (119).

The modulatory activity of quercetin on MAPK signaling was evaluated in several tumor cell lines. In the human hepatoma HepG2 cell line the treatment with quercetin induced a strong suppression of Akt and ERK1/2 phosphorylation but did not affect the expression of PI3K. In addition, quercetin inhibited NF-κB activation and upregulated the AP-1/JNK pathway. The modulation of these different signaling cascades induced apoptosis by direct activation of the intrinsic way (12,121). Conversely, quercetin arrested cell proliferation and induced apoptosis in A549 lung cancer cells, in a dose dependent manner, through the inactivation of Akt-1 and the enhancement of ERK-MEK1/2 phosphorylation (122).

Resveratrol, another polyphenol, was shown to impair MAPK signaling as well. Resveratrol induced growth inhibition and apoptosis by the transient activation of MAPK and inhibition of pS6 ribosomal protein expression in MDA-MB-231 breast cancer cells (123). This polyphenol exhibited modulatory activities on MAPK not only in breast cancer cells. In this regard, several studies reported the ability of resveratrol to affect MAPK signaling in different types of cancer. Parekh *et al.* demonstrated that resveratrol suppressed the growth of liver HepG2 cancer cells by down-regulating cyclin D1, p38 kinase, Akt and Pak1 expression and activity. In addition, the treatment with resveratrol increased phospho-ERK1/2 levels, inducing the activation of apoptosis (124). In human epidermoid carcinoma A431 cells, resveratrol exerted its anti-proliferative activity by inhibiting cyclin D1 and MEK1, ERK1/2 signaling and down-regulating c-Jun expression, which led to alteration of AP-1 activity and suppression of cell proliferation (125). In addition, combinatorial effects of resveratrol and black tea polyphenols on regression of tumor growth were reported by George *et al.* in BALB/c mice bearing skin tumors. The combinatorial treatment resulted in a significant regression of tumor volume and number and this phenomenon was associated with the reduction of MAPK (p38, ERK1/2 and JNK1/2) activity (126).

EGCG and theaflavins also inhibited the proliferation of DU145 and LNCaP prostate cancer cells by modulating PI3K and MAPK pathways. In this regard, EGCG and theaflavins decreased PI3K and phospho-Akt levels and enhanced ERK1/2 expression (127).

Impairment of PI3K and MAPK signaling pathways by curcumin has been demonstrated in a study by

Sun *et al*. They showed that curcumin inhibited cell proliferation and induced cell cycle arrest in G1 phase (at 30 μM) and apoptosis (at 50 μM) in ErbB2/Skp2-over-expressing breast cancer cell lines (MDA-MB-231^{ErbB2} cells). In addition, curcumin repressed the phosphorylation of ERK and Akt, without affecting JNK and p38 phosphorylation (128). The effects of the same polyphenol have been investigated in ovarian cancer cells as well. Curcumin induced p53-independent apoptosis through the down-regulation of Akt and phospho-Akt and the activation of p38 kinase, without affecting ERK1/2 activity in HEY cells (129).

Other studies have investigated the antiproliferative and differentiating activities of 5,7-dimethoxycoumarin on murine (B16) and human (A375) melanoma cell lines. The 5,7-dimethoxycoumarin reduced cell proliferation in a time- and dose-dependent manner by blocking cell cycle in G0/G1 phase. Furthermore, it was found for the first time that this compound inhibited the enzymatic activity of the activated MEK1/2, leading to a decrease of ERK1/2 phosphorylation (14,130). Effects of polyphenols on ErbB receptors signaling pathway in cancer cells are summarized in Table 1.

9. POLYPHENOLS AND THE HH/GLI SIGNALING PATHWAY IN CANCER CELLS

HH signaling pathway plays an important role in the carcinogenesis of medulloblastoma, an aggressive tumor of the cerebellum (131). A study by Elamin and co-workers investigated the effects of curcumin on medulloblastoma cells, and in particular its effects on the HH pathway. The authors demonstrated that curcumin had dose-dependent anti-proliferative and cytotoxic effects on four different medulloblastoma cell lines (MED-1, MED-4, MED-5 and DAOY). In addition, curcumin was able to increase the percentage of cells at the G2/M phase of the cell cycle. They also reported that curcumin (40 μM) was able to down-regulate the expression level of SHH protein (down-regulation of 12.5-fold after 8h of treatment) and its direct downstream targets GLI1 and PTCH1. The level of GLI1 and PTCH1 decreased more than 5 and 2-fold respectively, as compared to the control untreated cells. Moreover, it was shown that curcumin inhibited Akt/NF-κB and β-catenin pathways and triggered apoptosis through the down-regulation of Bcl-2, a downstream effector of HH pathway (132).

Chondrosarcoma, which shows abnormal activity of the human Indian Hedgehog (hIHH) signaling pathway, is a primary bone tumor with a poor prognosis (133). A recent study by Tang *et al*. investigated the effects of EGCG on growth and apoptosis of chondrosarcoma cells. They assessed that EGCG was able to affect proliferation and to induce apoptosis of SW1353 and CRL-7891 human chondrosarcoma cells in a dose-dependent manner. Moreover, they demonstrated that EGCG inhibited the hIHH pathway in these cell lines, through the down-regulation of PTCH1 and GLI1 mRNA and protein levels in a dose-dependent manner. These results suggest that EGCG could be a promising therapeutic agent for chondrosarcomas (134).

Another report by Slusarz *et al*. investigated the effect of seven polyphenols on prostate cancer: apigenin, baicalein, curcumin, EGCG, genistein, quercetin, and resveratrol. These compounds were individually able to inhibit the *in vitro* growth of PC3 and LNCaP human prostate cancer cell lines (135). In addition they were individually able to inhibit the growth of the mouse prostate cancer cell line TRAMP-C2 and to reduce or delay prostate cancer growth *in vivo* in TRAMP mice, when fed in combination. Moreover, the authors investigated the *in vitro* effects of these seven compounds on the HH pathway in two different cell assays, employing TRAMP-C2 and SHH Light II cells. Curcumin, genistein, EGCG and resveratrol were able to inhibit the HH signaling pathway in both cell assays, through the down-regulation of basal GLI1 mRNA expression in TRAMP-C2 cells and through the down-regulation of GLI reporter activity in the SHH Light II cell line. Conversely, apigenin, baicalein and quercetin, were able to decrease GLI1 mRNA expression in TRAMP-C2 cells, but not the GLI reporter activity in the SHH Light II cell line. Thus, the authors proposed that the effects of these polyphenols on prostate cancer cells might result from inhibition of the HH pathway (136).

Cancer stem cells (CSCs) play a key role in the carcinogenesis and progression of prostate cancer (137). Recent studies have shown that the activation of the HH pathway is involved in the regulation of pancreatic CSCs (138,139) and that polyphenols have anti-cancer stem cells (CSC) effects (140,141). A study by Tang *et al*. investigated the effects of the treatment with EGCG in pancreatic CSCs. Their results showed that EGCG was able to inhibit the growth and invasion of pancreatic CSCs and to induce apoptosis in these cells. Moreover, EGCG inhibited the HH pathway through the suppression of SMO, PTCH1, PTCH2, GLI-1 and GLI-2 expression and through the inhibition of GLI transcriptional activity. In addition, EGCG inhibited the nuclear expression of GLI1 and GLI2 proteins and quercetin synergized with EGCG in inhibiting GLI transcriptional and TCF/LEF activities, leading to the inhibition of self-renewal capacity of pancreatic CSCs (142). Similarly, Zhang *et al*. investigated the effects of genistein on the stemness properties of pancreatic CSCs. Genistein was able to inhibit tumorsphere formation and colony formation of prostate cancer cells and to suppress tumorigenicity *in vivo*. Moreover, the authors demonstrated that these effects of genistein were due to the down-regulation of the HH pathway. In fact, they found that the HH pathway plays an important role in the maintenance of pancreatic CSCs and that genistein was able to affect this pathway by down-regulating GLI1 mRNA and protein levels in a dose-dependent manner. They also demonstrated by immunohistochemistry that the treatment with genistein decreased the GLI1 protein expression level in pancreatic cancer tissue. Thus, they concluded that genistein could be a therapeutic agent for its capacity to affect the HH pathway, leading to the loss of stemness properties of pancreatic CSCs (143). Effects of polyphenols on the

HH/GLI signaling pathway in cancer cells are summarized in Table 1.

10. POLYPHENOLS AND NF-κB SIGNALING PATHWAY IN CANCER CELLS AND ACTIVATED MACROPHAGES

Among the different mechanisms by which polyphenols exert their anti-cancer effects, modulation of NF-κB activity is one of the most analyzed. In addition, due to the important role of tumor microenvironment inflammation in determining proliferation and survival of malignant cells, the effect of polyphenols on macrophages and cells involved in inflammation was analyzed (144). Indeed, the ability of several flavonoids to inhibit NF-κB activation in activated macrophages has been reported (145). The flavonoid apigenin has been demonstrated to have an inhibitory effect on NF-κB pathway in lipopolysaccharide (LPS)-stimulated mouse macrophages. In particular, apigenin regulated NF-κB activity by reducing phosphorylation of Ser^{536} in the p65 subunit and inactivating the IKK complex (146). The inhibitory effect on NF-κB activity is also displayed by quercetin in LPS-induced RAW 264.7 macrophages, in which the treatment with this flavonoid caused a significantly inhibition of NF-κB/p65 nuclear translocation (147). In addition, quercetin has also been demonstrated to inhibit NF-κB activation in the same cells through stabilization of the NF-κB/IκB complex and IκB degradation (148).

The impairment of NF-κB activity by EGCG has been investigated by Gupta *et al*. in human epidermoid carcinoma A431 cells. The authors demonstrated the inhibition of cell growth and the induction of caspases-dependent apoptosis in a dose dependent manner by EGCG (10-40 μg/ml). Moreover, EGCG induced a decrease in nuclear translocation of NF-κB/p65 (149).

The effects of some other polyphenols on NF-κB activation have been investigated by Romier *et al.* in human intestinal Caco-2 cancer cells. Among polyphenols, only chrysin and ellagic acid inhibited NF-κB activity, while others, such as genistein and resveratrol, determined a significant increase of NF-κB activation. The authors also reported that chrysin inhibited NF-κB activation by suppression of IκB-α phosphorylation (150).

Garcia-Mediavilla *et al.* reported that flavones such as quercetin and kaempferol were able to inhibit NF-κB activation in human hepatocyte-derived Chang liver cells and to reduce cellular levels of phosphorylated IκB-α and IKKα proteins (151). Quercetin and genistein also inhibited the proliferation of MCF-7^{ErbB2} breast cancer cells and induced apoptosis through down-regulation of IκB-α phosphorylation and subsequent suppression of the nuclear translocation of NFκB/p65 and its phosphorylation (152).

Different studies have shown that ACNs impair the transcriptional activity of NF-κB. Although the molecular mechanism through which ACNs interfere with NF-κB are not completely understood, it is without doubt that these compounds can prevent the degradation of the NF-κB inhibitor IκB by inhibiting the activity of the IκB kinase complex IKK. A study by Hafeez *et al*., reported that delphinidin was able to inhibit the growth of human prostate cancer cells (PC-3 cells and androgen refractory human PCa22Rv1 cells) with an IC50 value of 50-90 μM. Delphinidin (30-180 μM) also induced caspases-dependent apoptosis in the same cells in a dose-dependent manner, through the decrease of phosphorylation of IκB kinase γ (NEMO) and of NF-κB inhibitory protein IκB-α. Moreover, delphinidin reduced phospho-NF-κB/p50 at Ser529and phospho-NF-κB/p65 at Ser536 in the nuclear fraction, which led to inhibition of NF- κB/p65 DNA binding activity. Finally, the administration of delphinidin to mice carrying prostate cancer cell tumor xenografts induced a significant reduction of tumor growth and NF-κB protein levels in tumor tissues (153,154). Similar results were obtained by Yun *et al.* in human colon cancer cells. Delphinidin impaired cancer cell growth (IC_{50}: 110 μM) and induced apoptosis (at doses of 30 to 240 μM). Moreover, this polyphenol inhibited the activation of IKKα and IκB-α phosphorylation in a dose-dependent manner and the constitutive activation of NF-κB (155).

Ding *et al*. studied the inhibition of NF-κB by C3G derived from blackberries, in a mouse model of skin carcinogenesis. Pretreatment of mouse epidermal cells with C3G inhibited TPA- and UVB-induced NF-κB and AP-1 activity. In addition, C3G suppressed TPA- and UVB-induced phosphorylation of p38, ERK, JNK in a dose-dependent manner. These molecular events led to the suppression of tumor cell growth and metastasis in nude mice (156). Furthermore, other studies reported the inhibition of the LPS-induced phosphorylation of IκB-α and nuclear translocation of NF-κB by C3G and cyanidin in mouse leukemic macrophage-like cells (157), and by C3G in a human monocyte/macrophage cell line (158). In agreement with these results, Hecht *et al*. confirmed that C3G and its aglycon form, cyanidin chloride, derived from freeze-dried black raspberries, were good inhibitors of NF-κB activity induced by the mutagenic and highly carcinogenic benzo[a]pyrene-7,8-diol-9,10-epoxide (B[a]PDE) (159).

Wang *et al*. investigated the effects of ACNs from black raspberries on the development of *N*-nitrosomethylbenzylamine (NMBA)-induced rat esophagus tumors. They provided evidence that diets containing freeze-dried black raspberries suppressed the development of (NMBA)-induced tumors in the rat esophagus and this anti-tumor effect was associated with the reduction of expression of NF-κB/p50 and COX-2 at tumor level (160).

Pozo-Guisado *et al*. focused their study on the effect of resveratrol in MCF-7 human breast cancer cells. They observed that resveratrol (50-150 μM) induced a decrease in Bcl-2 protein levels and the subsequent activation of apoptosis in this cell line in a dose-dependent manner. Moreover, the authors found that the down-regulation of Bcl-2 expression levels was associated to the inhibition of NF-κB (161).

Table 1. Biological effects of polyphenols on ErbB receptors, MAPKs, HH/GLI and NF-κB signaling pathways

Signaling Pathways	**Polyphenol**	**Biological effects**	**Ref.**
ErbB receptors	EGCG	↓ ErbB2 phosphorylation and activation; ↓ PI3K/Akt, NF-κB	(68)
		↓ ErbB2-ErbB3 phosphorylation; ↓ PI3K/Akt, MAPK	(69)
		↓ EGFR, Stat3, Akt activity	(70)
		↓ ErbB2 phosphorylation; ↓Stat3, c-*fos*, cyclin D1	(71)
		↓ Akt activity	(73)
		↓ EGFR, ErbB2 phosphorylation	(75)
		↓ EGFR, ErbB2, ErbB3 phosphorylation	(76)
		↓ EGFR activation	(77-79)
	EGCG + ERLOTINIB	↓ EGFR, Akt, ERK1/2 phosphorylation	(80)
	EGCG + GEFITINIB	↓ EGFR phosphorylation; ↓phospho-ERK, -JNK, -p38, -Akt	(72)
	EGCG + DEXAMETHASONE	↓ Akt, NF-κB	(74)
	EGCG + U0216	↓ MAPK	(74)
	DELPHINIDIN	↓ EGFR, ErbB2 phosphorylation; ↓ ERK1/2 activity	(82)
		↓ EGFR, Akt, ERK1/2, JNK1/2, p38 phosphorylation	(83)
		↓EGFR, ErbB2 tyrosine kinase activity; ↓ ErbB3 phosphorylation	(84)
		↓ ErbB2, ERK1/2, Akt phosphorylation	(85)
	LIGNANS, SECORROIDS, GENISTEIN	↓ ErbB2 phosphorylation	(89,90,94,95)
	APIGENIN	↓ EGFR, ErbB2 phosphorylation	(12)
	LUTEOLIN	↓ EGFR phosphorylation; ↓ MAPK, PI3K/Akt activation	(87)
	QUERCETIN	↓ EGFR phosphorylation; ↓ ERK1/2 activity	(82)
		↓ ErbB2 tyrosine kinase activity; ↓ PI3K/Akt phosphorylation	(93)
	GENISTEIN + ERLOTINIB + GEFITINIB	↓ EGFR phosphorylation; ↓ Akt; ↓ NF-κB activation	(81)
	TANNIC ACID, ELLAGITANNINS, PROCYANIDINS	↓ EGFR phosphorylation	(88,91,92)
	LIGNANS AND SECORROIDS	↓ ErbB2 phosphorylation; ↓ Akt, MAPK, Stat3	(96)
	CURCUMIN	↓ ErbB2 tyrosine kinase activity	(97)
		↓ EGFR phosphorylation; ↓ c-*fos*; ↓ ERK, MKK4, JNK activity	(99)
		↓ EGFR, ERK1/2 phosphorylation	(100)
		↓ Akt, MAPK phosphorylation	(102)
		↓ ErbB2, phospho-Akt, phospho-MAPK, NF-κB	(103)
		↓ ErbB2	(104)
		↓ EGFR, ErbB2	(110)
		↓ EGFR, phospho-ERK1/2	(112)
		↓ Akt, phospho-ERK	(16)
	CURCUMIN + EGCG	↓ EGFR, Akt	(101)
	CURCUMIN + RESVERATROL	↓ EGFR, NF-κB	(105)
	CURCUMIN + DESATINIB	↓ EGFR, ErbB2, ErbB3; ↓ Akt, ERK phosphorylation; ↓ NF-κB	(106)
	CURCUMIN + FOLFOX	↓ EGFR, ErbB2, ErbB3; ↓ Akt phosphorylation	(107,108)
	CURCUMIN + BETULINIC ACID	↓ EGFR; ↓ Akt phosphorylation	(109)
	CURCUMIN + β-PHENILETYLISOTHIOCYANATE	↓ EGFR phosphorylation; ↓ PI3K/Akt activation; ↓ NF-κB	(113)
MAPKs	ANTHOCYANINS	↑ p38, ↓ Akt	(114)
		↓ ERK1/2 phosphorylation; ↓ AP-1	(115)
	APIGENIN	↑ JNK, ERK phosphorylation; ↓ p38, PI3K/Akt phosphorylation	(116,117)
		↓ p38, MAPK, PKA, PI3K/Akt	(118)
	FLAVANONE, 2'-OH FLAVANONE	↓ ERK1/2, p38 phosphorylation; ↓ AP-1, NF-κB activation	(119)
	QUERCETIN	↓ ERK1/2, Akt phosphorylation; ↓ NF-κB activation; ↑ AP-1, JNK	(120,121)
		↓ Akt activation; ↑ ERK-MEK1/2 phosphorylation	(122)
	GENISTEIN	↑ ERK, JNK phosphorylation	(116)
	KAEMPFEROL, NARINGENIN	↑ ERK, JNK phosphorylation	(116)
	RESVERATROL	↑ MAPKs activation	(123)
		↓ p38, Akt, cyclin D1, Pak1; ↑ phospho-ERK1/2	(124)
		↓ Cyclin D1, MEK1, ERK1/2, c-Jun	(125)
	RESVERATROL + BLACK TEA	↓ p38, JNK1/2, ERK1/2 activity	(126)
	EGCG, THEAFLAVINS	↓ PI3K/Akt; ↑ ERK1/2	(127)
	CURCUMIN	↓ ERK, Akt phosphorylation	(128)
		↓ Akt; ↓ p38 activation	(129)
	5,7-DIMETHOXY-COUMARIN	↓ MEK1/2 activity; ↓ ERK1/2 phosphorylation	(14,130)
HH/GLI	CURCUMIN	↓ SHH, GLI-1, PTCH1; ↓Akt, NF-κB	(132,136)
	EGCG	↓ hIHH, PTCH1, GLI-1	(134,136)
		↓ SMO, PTCH1, PTCH2, GLI-1, GLI-2	(142)
	APIGENIN, BAICALEIN, QUERCETIN, RESVERATROL	↓GLI-1	(136)
	GENISTEIN	↓ GLI-1	(136,139)
NF-κB	APIGENIN	↓ NF-κB phosphorylation; ↓ IKK activation	(146)
	QUERCETIN	↓ NF- κB/p65 nuclear translocation	(147)
		↓ NF- κB activation	(148)
	EGCG	↓ NF- κB activity and nuclear translocation	(149)
	QUERCETIN, GENISTEIN	↓ NF- κB nuclear translocation	(152)
	CHRISIN, ELLAGIC ACID, QUERCETIN, KAEMPFEROL	↓ NF- κB activity	(150,151)
	DELPHINIDIN	↓ Iκ-γ, Iκ-α phosphorylation; ↓ NF-κB/p65 DNA binding activity	(153,154)
		↓ IKK-α activation; ↓ Iκ-α phosphorylation; ↓ NF- κB activation	(155)
	C3G	↓ NF- κB, AP-1 activity; ↑ p38, ERK, JNK phosphorylation	(156,159)
	BLACK RASPBERRIES ANTHOCYANINS	↓ NF- κB/p50	(160)
	RESVERATROL	↓ NF- κB, Bcl-2	(161)
	CURCUMIN	↓ Iκ-α phosphorylation and degradation; ↓ NF- κB activation; ↓ AP-1, COX-2	(162)
		↓ NF- κB activation; Iκ-α degradation; ↓ NF- κB nuclear translocation; ↓ ERK1/2	(163)

Curcumin was shown to modulate NF-κB activity as well. Dyvia *et al*. studied the effects of curcumin in human papillomavirus (HPV)-associated cervical cancer cells. Curcumin induced apoptosis in these cells and blocked Iκ-α phosphorylation and degradation, leading to the inhibition of NF-κB activation. In addition, curcumin down-regulated AP-1 and COX-2 (162). Chun *et al*. assessed the effects of curcumin on mouse skin tumorigenesis *in vivo*. In particular, the authors investigated the effects of this polyphenol on TPA-induced expression of NF-κB and COX-2 in female ICR mouse skin. Curcumin inhibited the expression of COX-2 protein and the

activation of NF-κB in a dose-dependent manner. The inactivation of NF-κB was due to the inhibition of IκB-α degradation and the subsequent translocation of NF-κB/p65 into the nucleus. Furthermore, curcumin was also able to inhibit the catalytic activity of ERK1/2 in mouse skin (163).

Effects of polyphenols on NF-κB signaling pathway in cancer cells and activated macrophages are summarized in Table 1.

11. PERSPECTIVE

Polyphenols are natural compounds which have several biological activities and could be used as therapeutic agents to treat various human diseases, including cancer. Due to their ability to modulate the activity of multiple targets and receptors involved in carcinogenesis through simultaneous interaction or modulation of gene expression, polyphenols are considered "dirty drugs" (5,6). Neoplastic transformation is mediated by the modulation and interaction of different signal transduction pathways, that confer to cancer cells the ability to grow without exogenous stimuli, and determine the acquisition of aggressive tumor phenotypes by promoting neoangiogenesis and metastasis (1). Among the signal transduction pathways whose cross-talk plays an important role in neoplastic transformation are those mediated by ErbB receptors, NF-κB and the Hedgehog (HH)/glioma-associated oncogene (GLI) cascade. In this regard, a complex interplay can occur between ErbB receptors, HH signaling and NF-κB, and between HH and NF-κB signaling, and cooperation between EGFR signaling pathway and HH/GLI signaling cascade was demonstrated to promote cell transformation and proliferation of cancer cells. In this context, the PI3K/Akt and MAP kinase cascades are at the crossroads between the cooperative EGFR/ErbB2 receptors-HH/GLI signaling pathways (36,37).

The anti-tumor effect of polyphenols have been attributed to the inhibition of cell proliferation and to the induction of apoptosis in several tumor models both *in vitro* and *in vivo*, through the interaction with ErbB family receptors, MAPK, HH/GLI and NF-κB signaling pathway.

Despite promising results obtained from *in vitro* studies, the use of polyphenols as anti-cancer agents is yet limited in the clinical practice. However, there are several ongoing and completed clinical trials providing evidence of a safe and efficient use of polyphenols as anti-cancer agents (164-166).

The main drawback related to the use of polyphenols as drugs is their low bioavailability in the human body. However, compositions and methods for enhancing polyphenols bioavailability have been provided in the last twenty years (6).

Thus, depending on the development of polyphenols with better bioavailability and anti-cancer activities, these plant derivatives are promising drugs to design new clinical trials combining them with conventional therapies such as chemotherapy, radiotherapy and biological drugs which target those factors responsible of cancer progression.

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