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Lower concentrations of curcumin inhibit Her2-Akt pathway components in human breast cancer cells, and other dietary botanicals potentiate this and lapatinib inhibition

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ABSTRACT

Her2-dependent breast cancer is treated with pharmacological drugs (eg, Herceptin, lapatinib) that target Her2 signaling. Curcumin has emerged as a potential co-treatment for this and other cancers, but prior studies have focused on non-attainable concentrations. Here we test the hypothesis that attainable *in vivo* levels of dietary curcumin can reduce Her2 signaling. Consistent with previous studies, higher dose curcumin (18 μmol/L) inhibits Her2-Akt pathway signaling (pHer2, total Her2 and pAkt levels) and cell growth using AU565 human breast cancer cells. We then examined lower, more physiologically relevant concentrations of curcumin, alone and in combination with other dietary botanicals (quercetin and OptiBerry fruit extract). At 4 μmol/L, curcumin reduced Her2 signaling, and even more when combined with quercetin or OptiBerry. At 1.5 μmol/L curcumin, pHer2 and Her2 (but not pAkt) were reduced, with all three pathway markers reduced more in the presence of quercetin. We also found that 1.5 μmol/L curcumin strongly potentiated lapatinib inhibition of Her2-Akt pathway signaling, and more so for pAkt, when combined with quercetin plus OptiBerry (CQO). Parallel analyses revealed cell growth inhibition at 18 and 4 μmol/L but not 1.5 μmol/L curcumin, and potentiation of 1.5 μmol/L curcumin growth arrest with other botanicals +/– lapatinib. These studies demonstrate that a physiological attainable level of curcumin (1.5 μmol/L) can reduce some components of the critical Her2-Akt pathway; that even more complete inhibition can be achieved by combination with other dietary botanicals; and that curcumin and other botanicals can potentiate the action of the Her2-cancer metastatic drug lapatinib, in turn suggesting the potential anti-cancer clinical use of these botanicals.

Abbreviations: Her2, human epidermal growth factor receptor 2; Akt, serine/threonine-specific protein kinase; pHer2, phosphorylated Her2; pAkt, phosphorylated Akt; Cur, curcumin; Que, quercetin; CQO, curcumin plus quercetin plus OptiBerry; SEM, standard error of the mean.

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1. Introduction

Cancer is the second leading cause of death in the United States and is also among the leading causes of death worldwide [1-3]. Each year, more than half a million Americans die from cancer and predictably, incur high cost of care. In the United States, breast cancer is the most common cancer diagnosis after skin cancer in women, and is also the second leading cause of cancer death in females [2]. Thus, new approaches into breast cancer treatment and prevention are of major importance in health care. Her2-dependent breast cancer is a particularly aggressive form of this disease causing approximately 20–25% of breast cancers as well as contributing to ovarian, stomach, salivary duct, uterine and lung cancers [3-5]. It is the result of amplification of the *Her2* (*ERBB2*) gene, which encodes an epidermal growth factor receptor involved in cell proliferation that leads to phosphorylation activation of the Akt pathway (among others) and uncontrolled cell growth. Since this cancer is the result of an amplification of the *Her2* gene, its cause is clearly identified and by extension, is a logical target for treatment. This, in fact, has been exploited through the use of Herceptin (trastuzumab) and other drugs (eg, lapatinib). While pharmacological drugs in general have provided much benefit, they can be expensive, have side-effects, not benefit all patients, be limited in duration, and lead to resistance [5-9]. Many women with breast cancer look to improve their chances of survival post-conventional treatment (chemotherapy, radiation, surgery) by adding healthy lifestyle changes including diet.

We have proposed a low-cost and healthy treatment approach for numerous pathologies and conditions that we call "dietary rational gene targeting" [10,11]. Here, healthy dietary foods or supplements are used to modulate the expression of disease-causing genes back toward the normal. This strategy can potentially slow the disease process while lowering treatment cost and toxicity and is highly translatable since it simply requires diet modification. Other studies have also considered diet as a treatment for Her2-dependent breast cancer and other cancers and pathologies. Here, reduction of Her2 and/or Her2-signaling (often downstream pAkt levels) in cultured human Her2-overexpressing breast cancer cells has been reported with the botanicals curcumin, mangosteen, black pepper piperine, olive oil, silybin, and apigenin [5,12,13]. Regarding curcumin, numerous *in vitro* and rodent *in vivo* studies have provided evidence for curcumin anticancer activity including breast [14-20]. In addition, curcumin potentiation of chemotherapeutic agents and radiation therapy has also been studied [21,22]. Since these only involve a handful of studies, the extent of curcumin use in treating human breast and other cancers including breast is unclear and currently under investigation. Nonetheless, in addition to above, *in vivo* mouse studies show that curcumin reduces Her2 mRNA levels by approximately 3-fold [23], 32% [24], and decreases Her2-overepressing human xenograft size [22]. Additionally, numerous *in vitro* and rodent *in vivo* studies have shown curcumin ability to suppress tumor metastasis (including in mice injected with triple negative breast cancer cells) by modulating the expression of key driver genes, most notably VEGF-A and matrix metalloproteinases [15], supporting use of this dietary botanical for treating many other tumor types beyond just Her2-postive breast cancer. Curcumin is generally considered to be a safe nutraceutical and clinical trials have shown good tolerability and safety profiles at doses up to 12 grams per day [18,25,26].

One major drawback of using curcumin and other plant polyphenols to treat various pathologies is their low bioavailability, with many *in vitro* studies using concentrations well above those attainable in human plasma [27-31]. Here, we address this issue by testing the hypothesis that an attainable *in vivo* level of dietary curcumin (1.5 μmol/L) [32-34] can reduce Her2 signaling. We test this hypothesis by assessing the effect of this concentration of curcumin on Her2-Akt pathway signaling, cell proliferation, and inhibition by the Her2-cancer treatment drug lapatinib. We also assess this concentration of curcumin in combination with other plant polyphenols, as would be present during a typical healthy meal, to see if even greater pathway inhibition can be attained.

2. Methods and materials

2.1. Cell cultures

Human AU565 breast cancer cells [35] were cultured in DMEM supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 μg/mL streptomycin. All these reagents were obtained from Sigma Chemical Co (St Louis, MO, USA). Cultures were maintained in a humidified incubator atmosphere of 95% air and 5% CO_2 at 37 °C. AU565 cells used for these studies were between passages 106 and 113.

2.2. Cell culture treatments

Curcumin, quercetin and lapatinib were all obtained from Sigma Chemical Co and dissolved in DMSO (Sigma). OptiBerry, a mixture of six berry extracts [36-39], was obtained from InterHealth, (Benicia, CA, USA) and the dried extract first dissolved in DMSO for 5 minutes at room temperature before centrifuging 2 minutes at 12000*g* to obtain the soluble supernatant fraction for our studies. For treatment studies, cells were passaged (what passages?) into 60 mm cell culture plates. The next day, cultures were approximately 45% confluent and treated. For curcumin, CQO, and lapatinib treatments, agents were added directly to the cultures in whole media, and at the same time in the case of combination treatments. Equal volume DMSO only was used for control cultures. The total DMSO concentration for all cultures was 0.2% (v/v).

2.3. Western blot analyses

At the appropriate times after treatment, monolayer cell cultures were removed from the incubator, washed with PBS, lysed directly in the culture dish in standard 2× SDS sample buffer [11,40] containing 5% beta-mercaptoethanol, drawn through a 23 gauge needle repeatedly, and boiled for 4

minutes. Equal volume lysates were then electrophoresed on a 9% SDS-polyacrylamide one-dimensional gel, electroblotted to nitrocellulose (0.45 μmol/L; Bio-Rad, Hercules, CA, USA), preblocked in 5% non-fat dry milk (Cell Signaling, Danvers, MA, USA) and incubated with various antibodies as we have described [11,41,42]. These included antibodies to pHer2 (obtained from Cell Signaling) in 5% BSA (Cell Signaling) in TBST (Tris-buffered saline containing 0.1% Tween-20), total Her2 (Cell Signaling) in non-fat dry milk in TBST, pAkt (Santa Cruz Biotechnology, Dallas, TX) in 5% BSA (Cell Signaling) in TBST, and actin (Sigma) in non-fat dry milk in TBST. After primary antibody incubation and TBST washes, immunoblots were incubated with a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody followed by chemiluminescence signal development using the Western lightning plus kit substrate (PerkinElmer, Waltham, MA, USA) and signal capture with the ChemiDoc Touch imaging system (Bio-Rad). Antibody probings were successive and separated by blot stripping using the Restore Plus Western blot stripping buffer according to the manufacturer (ThermoFisher, Waltham, MA, USA).

2.4. *Effects of treatments on cell growth and viability*

Cells were plated in 48 well plates using 0.5 mL media and treated at approximately 45% confluence with and without phytochemicals and lapatinib as described above. After 36 hours, cells were trypsinized (Corning Incorporated, Corning, NY, USA) and counted. Aliquots were also mixed with an equal volume of 0.4% trypan blue solution (Sigma) and viability assessed microscopically as we have previously described [42,43].

2.5. *Statistical Analyses*

Western immunoblot signal and cell proliferation counts are expressed as the means +/– SEM (standard error of the mean). In comparing experiments, each result was normalized to controls, which were set at 100%. In the case of immunoblots, the effects of botanicals and/or lapatinib on Her2-Akt signaling were first normalized to actin and results then compared with solvent only controls using the Student *t* test. Statistical significance was concluded when $P \leq .05$ for any comparison.

3. Results

3.1. *18 μmol/L curcumin reduces AU565 cell Her2-signaling*

AU565 cells were treated for 6 and 20 hours with either 18 μmol/L curcumin, 25 μmol/L quercetin, the Her2 breast cancer treatment drug lapatinib (2 μmol/L) as a positive control, or solvent control (DMSO). Cell lysates were then immunoblotted using, sequentially, antibody to pHer2 (a marker of activated Her2), pAkt (a marker of activated Akt, a key proliferative down-stream pathway component of Her2 signaling), actin loading control, and total Her2. Results (Fig. 1; N = 3–5) show a significant reduction in pHer2, pAkt and total Her2 levels at both 6 and 20 hours with curcumin, and for 20 h pAkt with quercetin. The positive control, lapatinib reduced pHer2 and pAkt but not total Her2, actually increasing the latter. Based on the similar Her2-Akt pathway responses at 6 and 20 hours, future studies were conducted at just 20 hours.

3.2. *Curcumin also reduces AU565 Her2 signaling at 4 μmol/L*

Since 18 μmol/L curcumin is well above physiological levels achievable in blood plasma, we examined lower curcumin levels (4 and 1.5 μmol/L). At 4 μmol/L, curcumin reduced pHer2, total Her2 and pAkt levels (Fig. 2A; N = 4), similar to what we observed with 18 μmol/L curcumin.

3.3. *Curcumin reduction of AU565 Her2 signaling at 4 μmol/L is potentiated by other dietary botanicals*

In vivo, other dietary components and polyphenols might affect the extent of curcumin inhibition of Her2-Akt pathway signaling. Such an effect could potentially be exploited in patients with this cancer. To assess this, we combined curcumin with quercetin (4 μmol/L) and separately, OptiBerry (2 μg/mL) and repeated our analyses. Neither quercetin nor OptiBerry by themselves statistically reduced Her2-Akt signaling at these concentrations. However, when combined with 4 μmol/L curcumin, enhancement of curcumin reduction of both pHer2 and Her2 but not pAkt was observed (Fig. 2A "Curcumin plus quercetin"). When combined with OptiBerry, curcumin plus OptiBerry strongly inhibited pHer2, Her2 and pAkt (Fig. 2B; N = 4). The potentiating inhibitory effects of both quercetin and OptiBerry is additionally interesting since these agents do not inhibit Her2-Akt signaling by themselves, and suggests they act through some kind of synergistic interaction with curcumin. Overall, these results provide evidence that other dietary botanicals can potentiate curcumin inhibition of the Her2-Akt pathway.

3.4. *Inhibition of the Her2-Akt pathway is observed at attainable in vivo curcumin concentration (1.5 μmol/L)*

Curcumin concentrations as high as 3.8 μmol/L have been reported in blood plasma following dietary supplementation with this agent [32-34]. To assess the effect of curcumin in this concentration range on Her2-Akt signaling, we assessed 1.5 μmol/L curcumin. Similar to what we observed with 4 μmol/L and 18 μmol/L curcumin, we again saw reduction of pHer2 and Her2 but conversely, no reduction of pAkt (Fig. 3; N = 3). These results indicate that a lower, more physiologically relevant level of curcumin can reduce some but not all components of the critical Her2-Akt pathway needed for Her2-dependent cancer cell growth.

3.5. *Curcumin reduction of AU565 Her2 signaling at 1.5 μmol/L is potentiated by quercetin*

Similar to what was observed at 4 μmol/L curcumin, quercetin combined with curcumin (in this case, 1.5 μmol/L) led to a greater reduction of pHer2 and Her2 (Fig. 3). Interestingly, quercetin also led to a clear reduction in pAkt levels, something that was not observed for 1.5 μmol/L curcumin

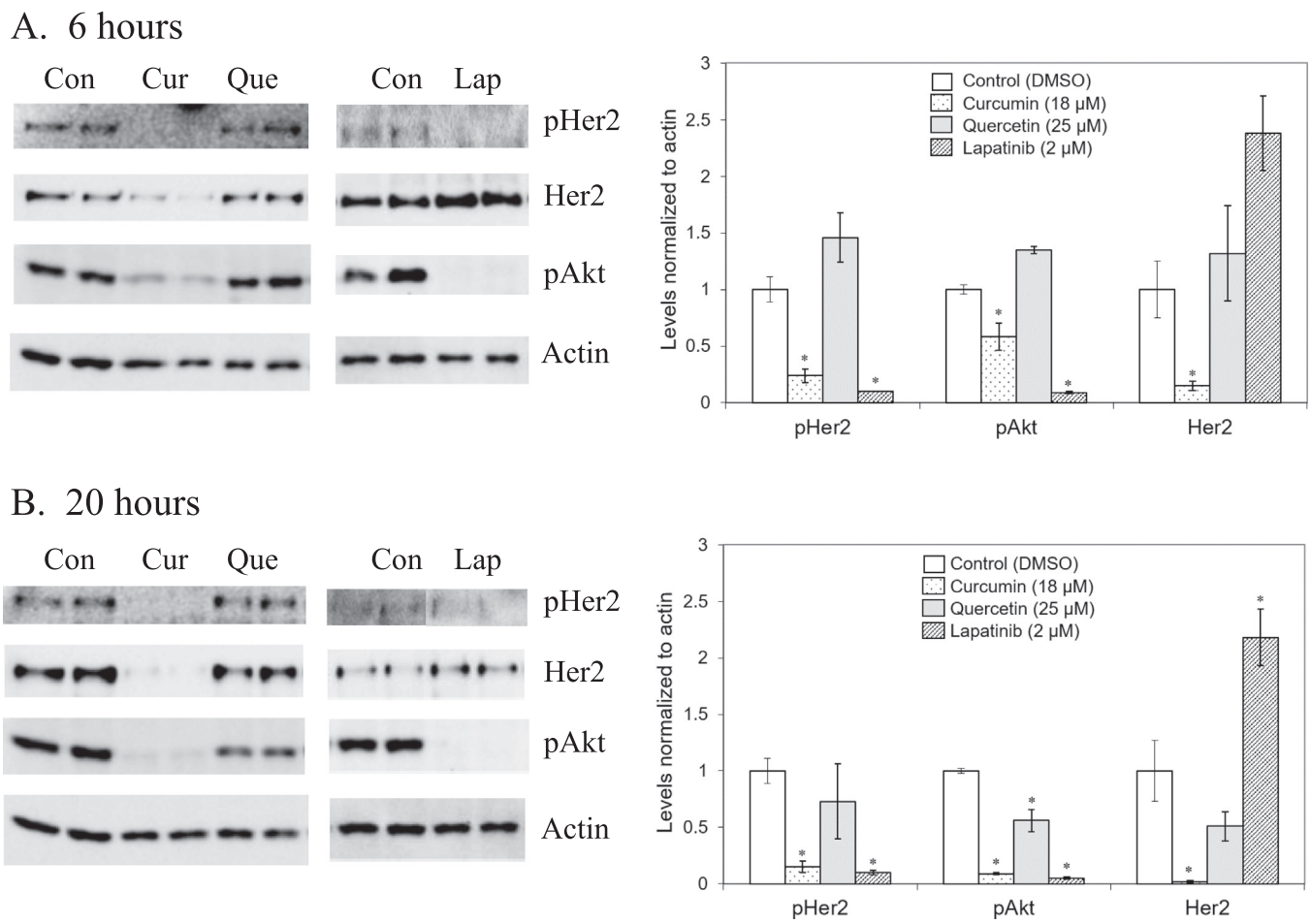

Fig. 1 – μmol/L), quercetin and lapatinib effects on AU565 cell Her2-signaling. AU565 cells were treated for the indicated times with 18 μmol/L curcumin, 25 μmol/L quercetin, 2 μmol/L lapatinib or solvent control (DMSO). Cell lysates were then immunoblotted using antibodies to pHer2, pAkt, Her2 and actin as a loading control. Representative immunoblots and plots after (A) 6 hours and (B) 20 hours treatment are shown. Plots were first normalized to actin. Data expressed as means +/– SEM (N = 3–5). *, significant difference compared with controls using the Student *t* test at *P* < .05. Cont, control; Cur, curcumin; Que, quercetin; Lap, lapatinib.

alone (Fig. 3; N = 3) or for 4 μmol/L curcumin plus quercetin (Fig. 2). These results indicate that even more complete inhibition of the Her2-Akt pathway can be achieved by combining physiologically attainable curcumin concentration with another dietary botanical; in this case, quercetin.

3.6. *Curcumin enhances lapatinib inhibition of the Her2-Akt pathway*

The pharmaceutical drug lapatinib is used as a treatment for Her2-positive breast- and other- cancers. It is especially useful for treating metastatic Her2-positive breast cancer that has become resistant to Herceptin (trastuzumab) therapy [44]. To determine whether curcumin also has potential as an adjunct treatment with other pharmaceutical treatment drugs, we assessed its impact on lapatinib inhibition of the Her2-Akt pathway. First, a lapatinib response concentration curve was carried out using a range of duplicate lapatinib concentrations (Fig. 4). We then selected two lapatinib concentrations that were in the range of 50% inhibition of pHer2 and pAkt. These included 20 nmol/L lapatinib (48.4% reduction of pHer2 levels as compared with solvent control, and 47.0% reduction of pAkt) and 5 nmol/L lapatinib (34.8% reduction of pHer2 levels as compared with solvent control, and 45.0% reduction of pAkt). 1.5 μmol/L curcumin was then combined with these concentrations of lapatinib. Indeed, at both concentrations of lapatinib, curcumin was found to potentiate inhibition as compared with lapatinib alone (Fig. 5; N = 4). Specifically, we observed statistically significant reductions for pHer2, pAkt, and total Her2 as compared with 5 nmol/L lapatinib alone (Fig. 5A), and a statistically significant reduction for Her2 and non-statistically significant reductions in pHer and pAkt as compared with 20 nmol/L lapatinib alone (Fig. 5B).

3.7. *Curcumin plus quercetin plus OptiBerry enhances lapatinib inhibition of pAkt levels*

We also assessed the effect of curcumin combined with other dietary botanicals (quercetin and OptiBerry) on lapatinib inhibition. This mixture, designated “CQO”, consisted of 1.5 μmol/L curcumin plus 4 μmol/L quercetin plus 2 μg/mL OptiBerry. The purpose of these studies was to determine

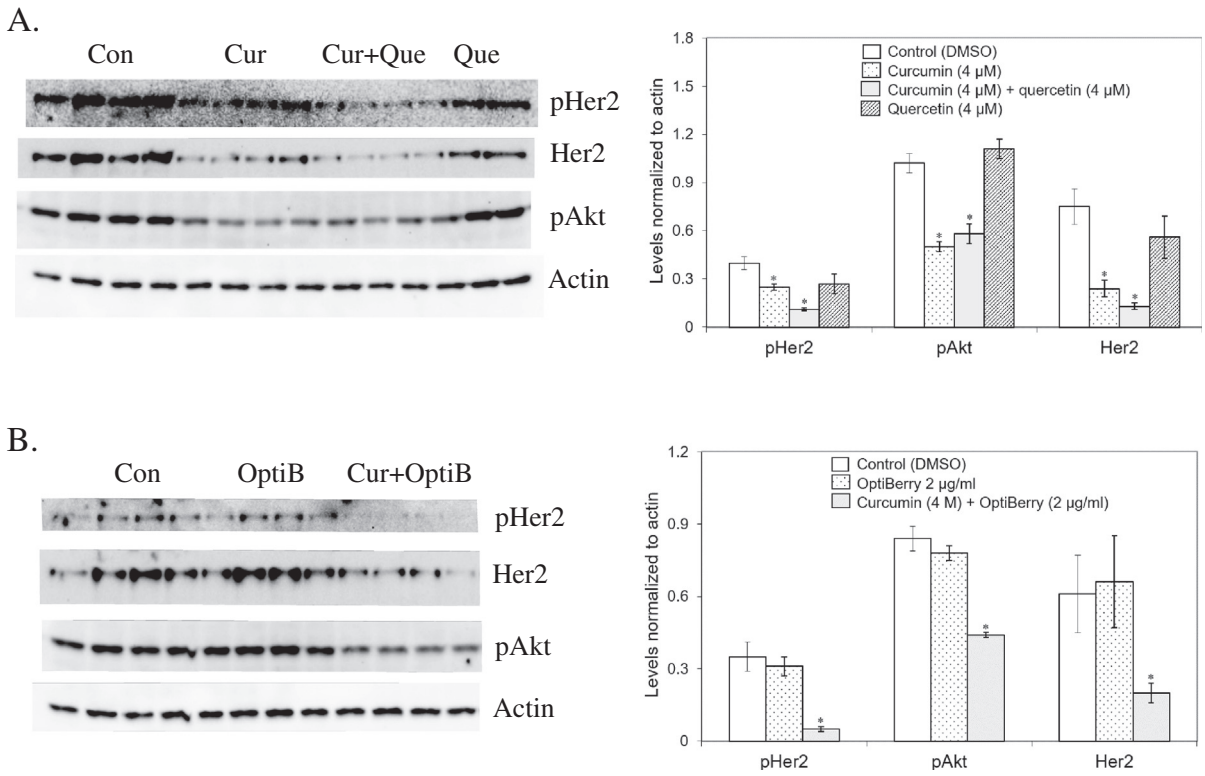

Fig. 2 – Curcumin (4 μmol/L), quercetin and OptiBerry effects on AU565 cell Her2-signaling. **AU565 cells were treated for 20 hours with 4 μmol/L curcumin, 4 μmol/L quercetin, 2 μg/mL OptiBerry or solvent control (DMSO) and cell lysates were then immunoblotted. Representative immunoblots and plots for (A) curcumin and quercetin treatments and (B) OptiBerry and combined curcumin plus OptiBerry treatments are shown. Plots were first normalized to actin. Data expressed as means +/– SEM (N = 4). *, significant difference compared with controls using the Student *t* test at *P* < .05. Cont, control; Cur, curcumin; Que, quercetin; OptiB, OptiBerry.**

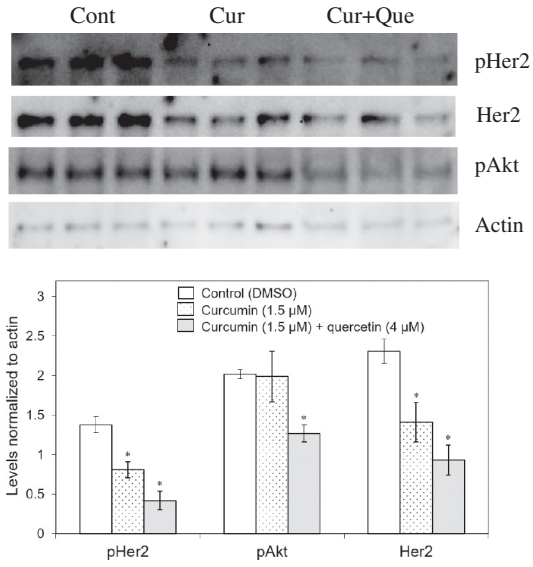

Fig. 3 – μmol/L) and quercetin effects on AU565 cell Her2-signaling. **AU565 cells were treated for 20 hours with 1.5 μmol/L curcumin, 1.5 μmol/L curcumin plus 4 μmol/L quercetin, or solvent control (DMSO) and cell lysates then immunoblotted. Plots were first normalized to actin. Data expressed as means +/– SEM (N = 3). *, significant difference compared with controls using the Student *t* test at *P* < .05. Cont, control; Cur, curcumin; Que, quercetin.**

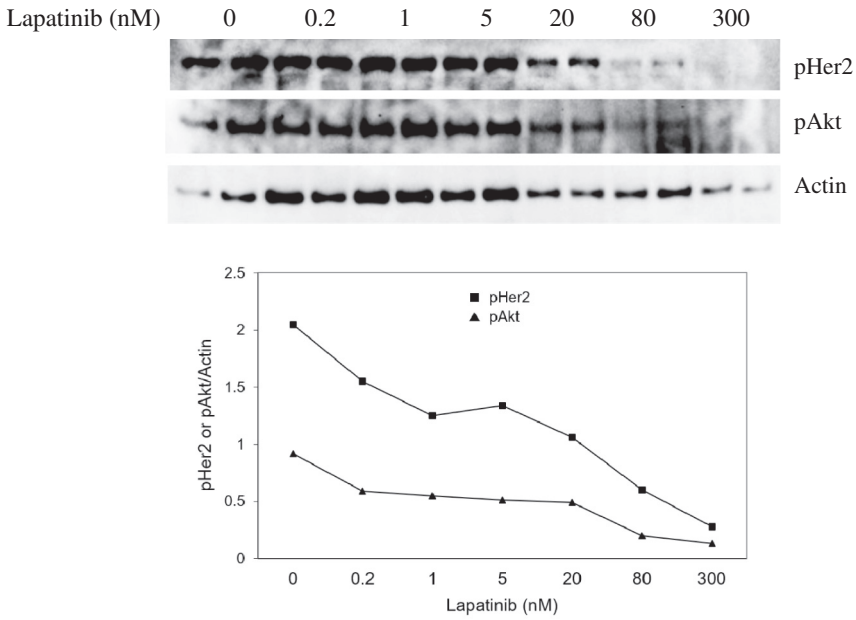

Fig. 4 – Lapatinib concentration effects on AU565 cell Her2-signaling. AU565 cells were treated for 20 hours with a range of lapatinib concentrations as shown or solvent control (DMSO) and cell lysates then immunoblotted. Plots were first normalized to actin. Data expressed as duplicate means. Cont, control; Cur, curcumin; Que., quercetin.

whether a healthy diet containing multiple botanicals and polyphenols might have the additional benefit of enhancing curcumin potentiation of lapatinib inhibition, including potentially in patients undergoing lapatinib therapy. At 5 nmol/L lapatinib, CQO potentiation of lapatinib inhibition of Her2-Akt signaling was near identical to that observed for curcumin alone (Fig. 5A), indicating no additional inhibitory effects by the quercetin and OptiBerry botanicals. At 20 nmol/L lapatinib, CQO potentiation of lapatinib inhibition of Her2-Akt signaling was again near identical to that observed for curcumin alone for pHer2 and total Her2 (Fig. 5B). However, CQO reduced pAkt levels beyond that observed with lapatinib plus 1.5 μmol/L curcumin only. In fact, CQO statistically reduced pAkt levels compared with ether control or lapatinib alone, neither of which was observed with curcumin only. Extending this comparison, we also observed a statistically significant greater pAkt/actin reduction with CQO when compared directly with curcumin only (both at 20 nmol/L lapatinib). These results indicate that for at least one component of the Her2-Akt pathway, other botanicals (here, quercetin and OptiBerry) can enhance the already observed curcumin potentiation of lapatinib inhibition. This also suggests that a plant-based diet might further potentiate curcumin effects on lapatinib inhibition.

3.8. *Curcumin and CQO both reduce lapatinib-induced Her2 levels*

In Fig. 1 studies, we noticed an absence of total Her2 level reduction with 2 μmol/L lapatinib despite strong reductions in pHer2 and pAkt. This has been reported by others as well [45, 46]. In fact, we observed a statistically significant increase in Her2 at both 6 and 20 hours with 2 μmol/L lapatinib when compared with control levels (Fig. 1). A statistically significant induction in Her2 levels was also observed with 20 nmol/L lapatinib (Fig. 5B). Since we are interested in reducing overall Her2-Akt signaling with diet, the effect of curcumin and CQO on these lapatinib increases in Her2 is also of interest. As shown in Figs. 1A, B and 5B, curcumin strongly reversed lapatinib-induced Her2 levels. The same was observed with CQO where studied (Fig. 5B), although its reversal was near identical to that observed with curcumin alone, suggesting that the CQO inhibition was due to its curcumin content. Nonetheless, these studies indicate that curcumin can strongly reverse lapatinib induction of total Her2 levels, and this action might represent yet another way by which curcumin acts as a lapatinib adjuvant.

3.9. *Curcumin and other botanicals exhibit some inhibition of AU565 cell proliferation*

We also assessed cell growth for all the above studies using N = 4–6 samples. For our initial studies, 18 μmol/L curcumin inhibited cell growth by 68% ($P \leq .05$ *vs* control) after 36 hours (Fig. 6A). Lesser but still statistically significant curcumin inhibition was also observed at 4 μmol/L but not 1.5 μmol/L curcumin (Fig. 6A). Individually, both quercetin and OptiBerry further reduced the cell growth observed at 4 μmol/L curcumin, although these effects were modest (Fig. 6B). For 1.5 μmol/L curcumin, combination with quercetin led to a modest but statistically significant inhibition of cell growth (Fig. 6C). However, this was also observed with quercetin alone and so does not appear to represent an additive effect. The lack of 1.5 μmol/L curcumin reduction of cell proliferation is likely explained, at least in part, but its lack of pAkt reduction despite clear pHer2 and Her2 reductions as shown in Fig. 3. This lack of a 1.5 μmol/L curcumin reduction in cell growth also underscores our earlier conclusion that 1.5 μmol/L curcumin by itself can reduce some components of the critical

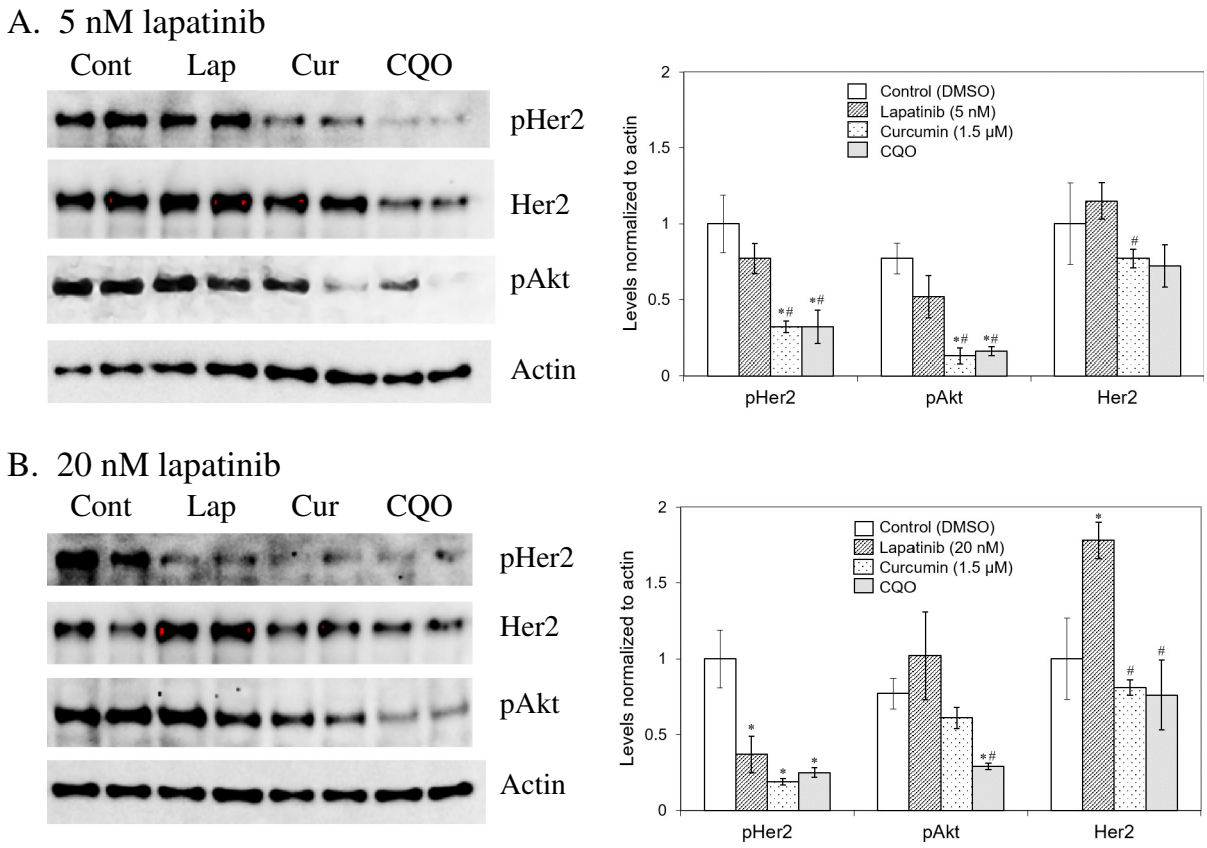

Fig. 5 – Effect of curcumin and CQO on lapatinib inhibition of AU565 cell Her2-signaling. AU565 cells were treated for 20 hours with (A) 5 nmol/L lapatinib or (B) 20 nmol/L lapatinib +/– 1.5 μmol/L curcumin or CQO and cell lysates immunoblotted. Representative immunoblots and plots after normalization to actin are shown. Data expressed as means +/– SEM (N = 4). *, significant difference compared with controls using the Student t test at P < .05. #, significant difference compared with lapatinib using the Student t test at P < .05. Cont, control; Cur, curcumin; Lap, lapatinib; CQO (1.5 μmol/L curcumin plus 4 μmol/L quercetin plus μmol/L 2 μg/mL OptiBerry).

Her2-Akt pathway (pHer2, Her2) but not all, providing more detailed insight into its potential contribution to treating Her2-dependent breast cancer, especially when considering its potentiation of lapatinib inhibition (Fig. 5 and below). For all these studies, concomitant trypan blue analyses did not reveal increased cell death for any treatment as compared with control, at least at this 36-hour time point.

3.10. *Curcumin and other botanicals also potentiate lapatinib inhibition of cell growth*

As described above, we first carried out a concentration curve to assess the effect of lapatinib. Cell growth analyses were also carried out at these lapatinib concentrations as well as at 2 μmol/L (used for the Fig. 1 study). Except for the latter, we did not observe any statistically significant reduction in proliferation, although a modest trend of reduced growth with increasing lapatinib concentration was observed (Fig. 7A; N = 4–6). When combined with botanicals, however, statistically significant growth inhibition was observed in some cases; specifically, when 5 nmol/L lapatinib was combined with CQO, and when 20 nmol/L lapatinib was combined with either 1.5 μmol/L curcumin alone or with CQO (Fig. 7B; N = 4–6). The enhancing effects of CQO on cell growth beyond the 1.5 μmol/L curcumin alone is also considerably more prominent than what we saw for Her2-Akt components in Fig. 5, and indicate that (1) other botanicals combined with curcumin have cancer cell treatment potential beyond that observed for curcumin alone, and (2) other pathways beyond just Her2-Akt contribute to AU565 cell growth inhibition (eg, MAP kinase). Furthermore, the actions of these compounds are shown in Fig. 8.

4. Discussion

In these studies, we assessed the effect of curcumin alone or in combination with other botanicals as a potential treatment for Her2-dependent breast cancer. Importantly, most of our analyses were carried out using 1.5 μmol/L curcumin. This concentration was selected because published studies report this range of concentration is attainable in human blood following curcumin supplementation. For example, Schiborr et al reported blood levels of 3.2 μmol/L curcumin in a micellar system in healthy humans [32]; Cheng et al found levels as high as 1.77 μmol/L in subjects with high risk cancer

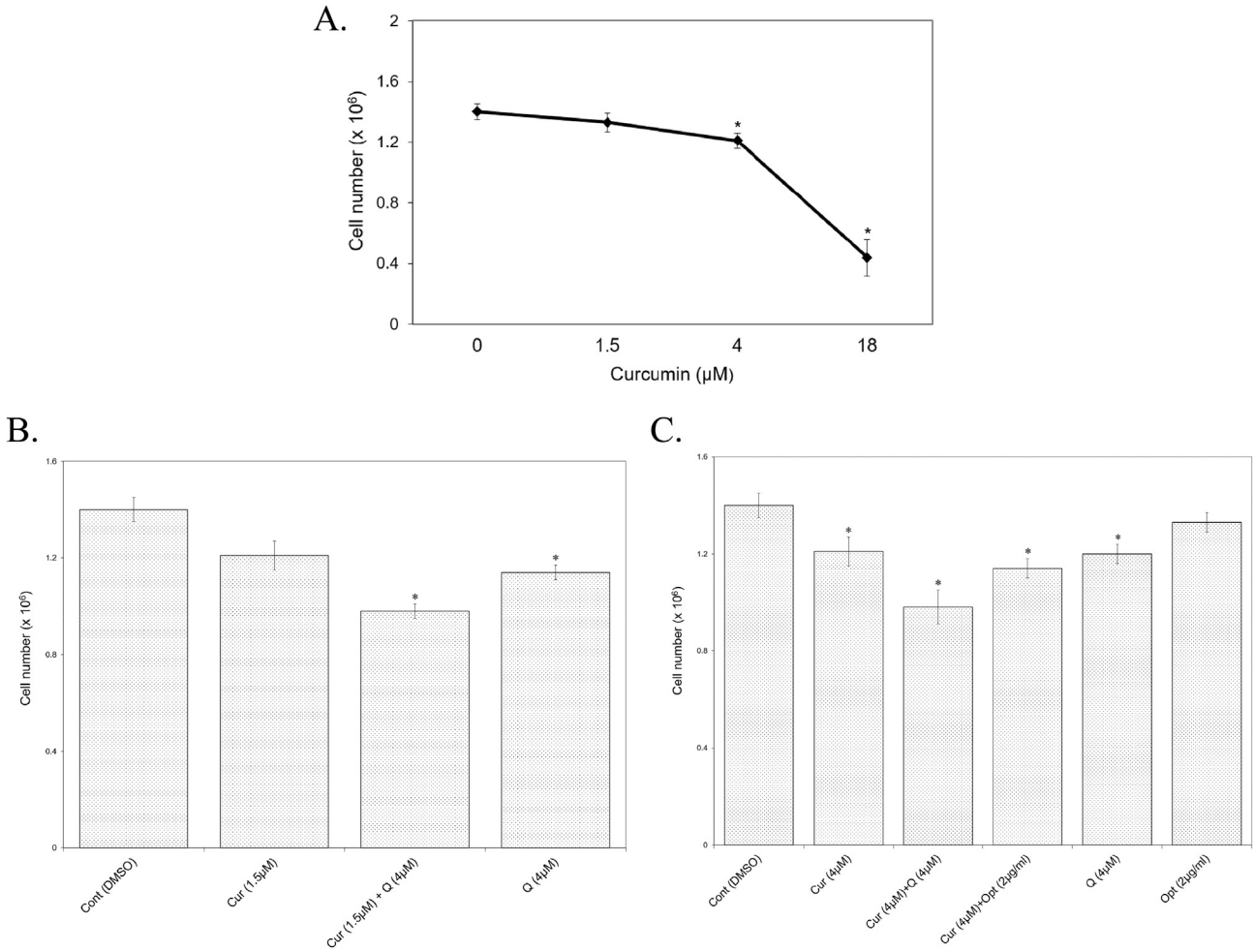

Fig. 6 – Effect of dietary botanicals on the growth of AU565 cells. AU565 cells were treated with (A) different concentrations of curcumin, (B) 4 μmol/L curcumin +/– 4 μmol/L quercetin or 2 μg/mL OptiBerry, and (C) 1.5 μmol/L curcumin +/– 4 μmol/L quercetin, and cells counted 36 hours later. Data expressed as means +/– SEM (N = 4–6). *, significant difference compared with (DMSO) controls using the Student *t* test at *P* < .05. Cont, control; Cur, curcumin; Q, quercetin; Opt, OptiBerry.

lesions following curcumin supplementation [33]; and Kanai et al found levels as high as 3.8 μmol/L (1.4 μg/mL) in cancer patients following Theracurmin supplementation [34]. At this attainable 1.5 μmol/L curcumin concentration, we observed successful inhibition of at least part of the critical Her2-Akt pathway that is so critical to Her2-positive cancer uncontrolled growth [47].

The issue of human *in vivo* curcumin (and other polyphenol) bioavailability has become a major consideration in evaluating the health benefits of dietary botanicals. In general, plant polyphenols have weak bioavailability. Thus, while many *in vitro* curcumin benefits have been reported (eg, anti-inflammation and antioxidation), translation to treating humans is questionable [27-31]. For this reason, and at this stage of research in the long-studied curcumin field, we believe that low concentration *in vitro* studies should be a focus such as we have carried out here. Most previous *in vitro* studies on curcumin, including anticancer studies, have used much higher concentrations that are well above these attainable human *in vivo* levels [eg, [48,49]]. This has been a major hurdle in translating cell culture studies to human *in vivo*. One notable exception for Her2-dependent cancer cell line studies is that of Lai et al who, while focusing mostly on higher curcumin concentration studies, also examined concentrations as low as 3 μmol/L in different cell lines although they did not examine pHer2 levels, combination with other nutraceuticals, combination with lapatinib, and a lower curcumin concentration as we have done here [22]. Nonetheless, despite low attainable plasma curcumin concentrations, it is encouraging that a growing list of clinical pathologies and conditions have shown benefit from curcumin administration as reported in 17 out of 49 curcumin double-blinded placebo-controlled clinical trials plus 27 other clinical trials [19,50].

Our studies also evaluated the combinatorial effects of other dietary botanicals and polyphenols with curcumin; specifically, quercetin, which is one of the most abundant flavonoid polyphenols in nature and present in many fruits and vegetables [51], and OptiBerry, a mixture of six berry extracts [36-39]. As presented above, we saw significant enhancement of curcumin inhibition of Her2-Akt signaling with these other botanicals. This suggests that curcumin supplementation is not only a potential treatment, or co-

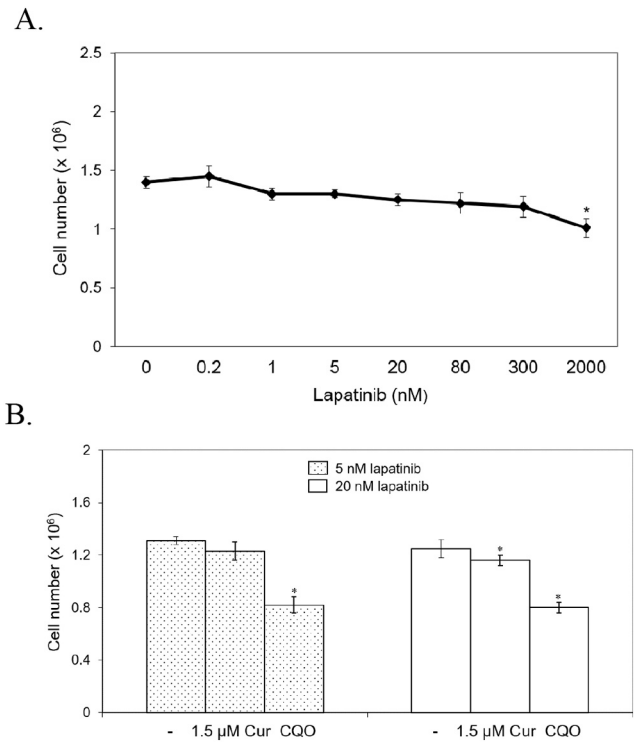

Fig. 7 – Effect of botanicals on lapatinib inhibition of AU565 cell growth. AU565 cells were treated with (A) different concentrations of lapatinib and (B) 5 nmol/L or 20 nmol/L lapatinib +/– 1.5 µmol/L curcumin or CQO. Data expressed as means +/– SEM (N = 4–6). *, significant difference compared with (DMSO) controls using the Student t test at $P < .05$. Cur, curcumin; CQO, curcumin plus quercetin plus OptiBerry.

adjuvant treatment for cancer patients, but also a beneficial anti-cancer effects can be enhanced in the presence of a plant-based diet. Such a strategy is the basis of our "dietary rational gene targeting" approach and provides an additional option for cancer patients that is much lower cost and healthier than conventional Her2-positive cancer treatments such as chemotherapy or Herceptin.

We also evaluated the effect of curcumin with and without the other botanicals in enhancing the efficacy of lapatinib. To our knowledge, this is the first time this has been assessed. There is growing interest in the combinatorial benefits of curcumin in improving the efficacy and safety of conventional treatment agents as well as Herceptin [21,22]. Lapatinib is a valuable drug for treating Her2-dependent breast cancer. Here, it is used both in combination with most notably Herceptin to treat Her2-dependent breast cancer, and by itself to treat metastasis in stage 3 or higher patients who no longer respond to treatment such as chemotherapy and Herceptin. Our objective here was to determine whether curcumin by itself or in combination with other dietary botanicals might enhance lapatinib inhibition of both Her2-Akt signaling and cell growth. Our observation that curcumin can indeed potentiate lapatinib action, and even more so in the presence of other botanicals and polyphenols, suggests that our botanical approach has promise for patients on lapatinib.

At this stage, there are two limitations to our studies. First, while we have focused on a key pathway (Her2-Akt) involved in Her2-dependent cancer, there are other pathways involved in the development of this cancer as well (e,g., MAP kinase) and so their response to our botanical treatments in human Her2-breast cancer cells would also be of interest. Second, we have used a native unformulated and unmodified form of curcumin for our studies but the use of this type preparation *in vivo* in human is questionable. For related studies, we cite Cheng et al [33] who used a similar preparation *in vivo* and achieved 1.7 µmol/L plasma levels, but other studies have reported much less plasma curcumin such that new curcumin formulations designed to drastically increase bioavailability look most promising at this time including for treating Her2-dependent breast cancer [32,34,52].

In summary, our studies provide evidence that physiological attainable levels of curcumin can inhibit Her2 signaling in Her2-dependent breast cancer cells; that other botanicals can potentiate this effect; and that curcumin and other botanicals also potentiate the action of the metastasis treatment drug lapatinib. Thus, we accept our research hypothesis that attainable *in vivo* levels of dietary curcumin can reduce Her2 signaling. Our studies also demonstrate that in some cases, partial rather than complete inhibition of Her2-Akt signaling and cell growth occurs and as such, provide more detailed insight into exactly what potential benefits physiologically attainable concentrations of curcumin might provide.

Declaration of competing interest

None

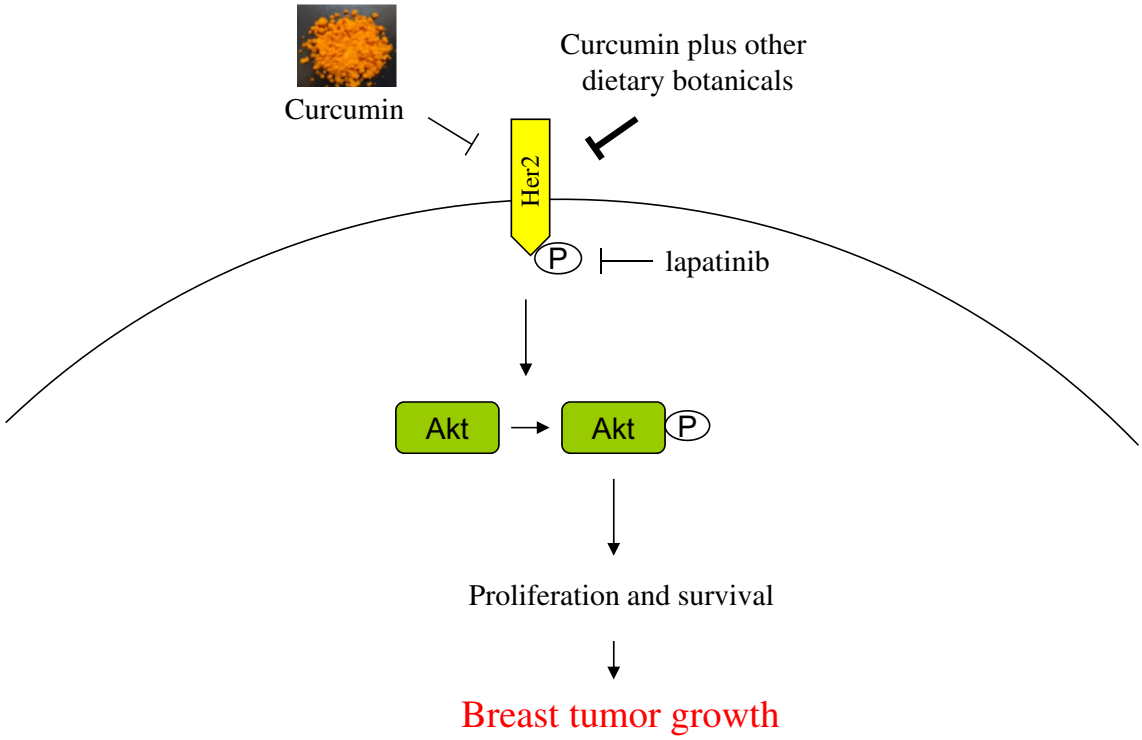

Fig. 8 – Proposed model. **Exposure of Her2-dependent breast cancer cells to curcumin, either experimentally or through dietary supplementation, inhibits the key Her2-Akt signaling pathway, in turn reducing cancer cell proliferation and survival. Even greater inhibition can be achieved by supplementing curcumin administration with other dietary botanicals. These dietary agents may inhibit Her2 signaling at a site different than lapatinib, leading to additive inhibitory effects.**

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