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14. ABSTRACT

The cancer preventive properties of individual grape and red wine polyphenols have been investigated. However, such studies focus mainly on individual compounds at concentrations too high to be achieved by dietary intake. In this study, we proposed to test the hypothesis that combined grape and red wine polyphenols at low concentrations can be more effective than individual compounds at inhibition of breast cancer progression. For this, we tested the effects of resveratrol, quercetin, and catechin, which constitute 70% of red wine polyphenols, on biological processes relevant to breast cancer progression using breast cancer cell lines. We found that combined grape polyphenols induce apoptosis and are more effective than individual resveratrol, quercetin, or catechin at inhibition of cell functions that promote breast cancer progression. Using estrogen receptor (ER)α(-), ERβ(+) MDA-MB-231 and ER(-) MDA-MB-435 breast cancer cell lines we demonstrated that combined grape polyphenols increased apoptosis and inhibited cell proliferation, cell cycle progression, and cell migration. Work in the laboratory also showed that combined grape polyphenols reduced primary breast tumor growth and metastasis in nude mice (Castillo-Pichardo, et al., 2009, Clin. Exp. Met. 26:505-16). Studies are underway to identify the molecular targets of grape polyphenols in breast cancer.

15. SUBJECT TERMS
Grape polyphenols, breast cancer, apoptosis, cell proliferation, cell migration, cell cycle progression

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Introduction

Breast cancer is the most commonly diagnosed form of cancer in women 40-55 years of age and is the second major cause of cancer deaths [1]. Metastatic breast cancer, where cancer cells spread by motile mechanisms and establish tumors at distant vital sites, is much harder to eradicate and is often the cause of death from breast cancer. The five year survival rate for metastatic breast cancer is only 26%. Therefore, it is important to investigate strategies for prevention of breast cancer metastasis. Most studies on environmental and dietary anticancer compounds focus on cancer initiation and not the impact of these compounds on metastatic cancer. This proposal is unique in evaluating a role for a dietary compound in breast cancer metastasis, the most deadly aspect of the disease. This timely research is **significant to public health** by advancing the understanding and treatment of breast cancer metastasis and **impact** breast cancer patients, those at risk, and survivors of breast cancer. The cancer chemopreventive action of grape products like red wine have been attributed to the antioxidant and antiproliferative properties of polyphenolic compounds. The proposed research will **investigate a novel signaling mechanism** by which estrogen (E_2) like polyphenols from grapes signal to affect breast cancer progression and metastasis using antibody microarrays. Much of the data on the cancer preventive effects of grape polyphenols has been generated from tissue culture cell lines treated with individual polyphenols at concentrations too high to be achieved via dietary consumption. Thus, the utility of polyphenolic compounds as dietary cancer chemopreventives is hampered by their bioavailability at target tissues following consumption. Therefore, prior to clinical development of these polyphenols as cancer chemopreventives it is essential to investigate their mechanism of action at a range of concentrations. **This research will identify signaling intermediates of dietary polyphenols with potential for breast cancer therapy.** The proposed research will not only impact therapeutic design to combat breast cancer but will also aid in understanding novel signaling mechanisms that directly regulate cell functions relevant for breast cancer metastasis. In addition to such contributions, this project will allow the PI to obtain a Ph.D. degree, which is integral for the pursuit of her long term career goals. The PI plans are to become a researcher and faculty member at a major research institution in order to impact the development of therapeutic targets for treatment and prevention of breast cancer.

Body

A. The following statement of work describes the PI's proposed **training plan**:

Task 1. Complete the second year of coursework
Status: Completed prior to this training grant start date.

Task 2. Complete the requirements for the candidacy to Ph.D. Degree

- **I.** Take comprehensive examination to gain admittance to Ph.D. candidacy.
- **II.** Write and present Thesis Proposal to Ph.D. Dissertation Committee.

Status: Completed

Task 3. Complete the Dissertation research for Ph.D. Degree

- **I.** Complete the independent research requirement for the Ph.D. Degree.
- **II.** Submit two manuscripts for publication in peer–reviewed journals.
- **III.** Write Ph.D. Dissertation and successfully defend Dissertation.

Status: Dissertation research in progress and one research article was published (see appendix).

B. The following statement of work describes the proposed **research plan** of testing the hypothesis that combined grape polyphenols regulate signal transduction to modulate breast cancer progression to metastasis:

Task 1. Determine the effective concentration for antibodies to be used in the study
Status: A commercially available antibody array that suited the purposes of the proposed project was found. Therefore *Task 1* was not necessary.

The cytoskeletal phospho-specific antibody microarray from Full Moon Biosystems, Inc. was used. This array includes 95 highly specific and well-characterized phosphorylation antibodies (see appendix for a detailed list) in the cytoskeletal pathway, which are important regulators of migration and invasion, and

therefore, of metastasis. The non-phospho pairs are also included to allow a determination of the phosphorylation state of each of these proteins.

Task 2. Determine the effect of individual grape polyphenols on mammary epithelial and breast cancer cells.

Task 3. Determine the effect of individual grape polyphenols on breast cancer cells.

Task 4. Determine the effect of combined grape polyphenols on breast cancer cells.

Status: Tasks 2-4 propose to delineate signal transduction from individual and combined grape polyphenols to proteins that regulate breast cancer metastasis using antibody microarrays. However, previous to elucidating the effects of grape polyphenols on signal transduction it was necessary to further characterize how these compounds influence biological processes relevant for breast cancer progression and metastasis. Therefore, we decided to study the effects of resveratrol, quercetin, and catechin individually or combined (RQC) at low dietary concentrations on breast cancer cell proliferation, cell cycle progression, apoptosis, and cell migration. The effects of individual and combined resveratrol, quercetin, and catechin on cell proliferation and cell cycle progression of the low metastatic, estrogen receptor (ER)α(+), ERβ(-) MDA-MB-231 cell line were presented on the preliminary data section of the original proposal narrative and have been published in [2].

Cell proliferation

Previously, we showed that in MDA-MB-231 cells, combined grape polyphenols were more effective than individual compounds at inhibition of cell proliferation and cell cycle progression causing an S to G2 phase arrest of the cell cycle [2]. We then decided to test the effects of grape polyphenols on the highly metastatic, ER negative MDA-MB-435 cell line to address the role of ER in the response to such compounds. MDA-MB-435 cells were treated with vehicle, 0.5, 5, or 20μM resveratrol, quercetin, catechin or RQC every 48h for 96h and cell number was quantified (Fig. 1). At 0.5μM, treatment with individual resveratrol or catechin did not cause significant changes in cell number, while quercetin caused an increase in cell number. Interestingly, RQC at 0.5μM significantly decreased cell proliferation by approximately 60%. At 5μM none of the individual grape polyphenols inhibited cell proliferation. However, 5μM RQC treatment decreased proliferation by an 80%. At 20μM only resveratrol, quercetin and RQC had an anti-proliferative effect at 20μM. From these results it can be concluded that, as previously shown with MDA-MB-231 cells [2], combined grape polyphenols at low concentrations are more effective than the individual compounds at inhibiting MDA-MB-435 breast cancer cell proliferation. However, MDA-MB-231 cells showed higher sensitivity to RQC.

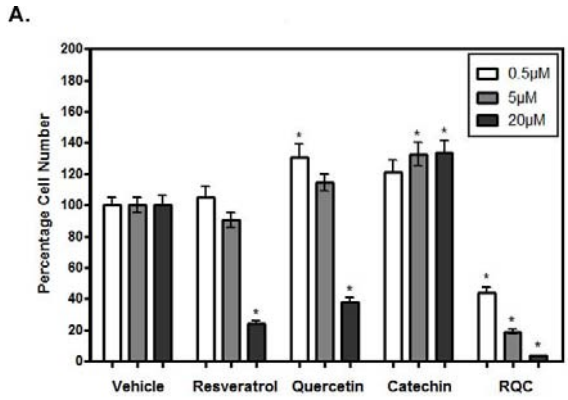


Figure 1. Effect of grape polyphenols on MDA-MB-435 cell proliferation. MDA-MB-435 cells in 5% serum and phenol red-free media were treated with vehicle, 0.5, 5, or 20μM resveratrol, quercetin, catechin, or RQC every 48h for 96h. Cell number was quantified from PI-stained intact (non-apoptotic) nuclei. **A**, Percentage of viable MDA-MB-231 cells ± SEM for 20 microscopic fields/triplicate treatments. An asterisk indicates statistical significance ($p \leq 0.05$) when compared to vehicle.

Cell cycle progression

MDA-MB-435 cells were treated with vehicle, 0.5 or 5μM resveratrol, quercetin, catechin or RQC every 48h for 96h. The effect of such treatments on cell cycle progression was determined by flow cytometric analysis of PI-stained cells (Fig. 2). While individual polyphenols did not show an effect on cell cycle progression, a moderate S phase arrest by RQC treatment at 5μM was observed (Fig. 2B). The S phase arrest of the MDA-MB-435 cells in response to RQC showed a $p \leq 0.06$, which is slightly less significant than the S phase arrest of $p \leq 0.05$ observed with MDA-MB-231 cells [2]. Again, combined grape polyphenols were proven to be more effective than individual compounds at inhibition of cell proliferation and cell cycle progression. However, the 80% decrease in MDA-MB-435 cell proliferation induced by 5μM RQC does not correlate with the observed modest increase in cells at S-phase. Therefore, we decided to investigate the effect of RQC treatment on apoptosis by measuring Annexin V staining and caspase 3 activity.

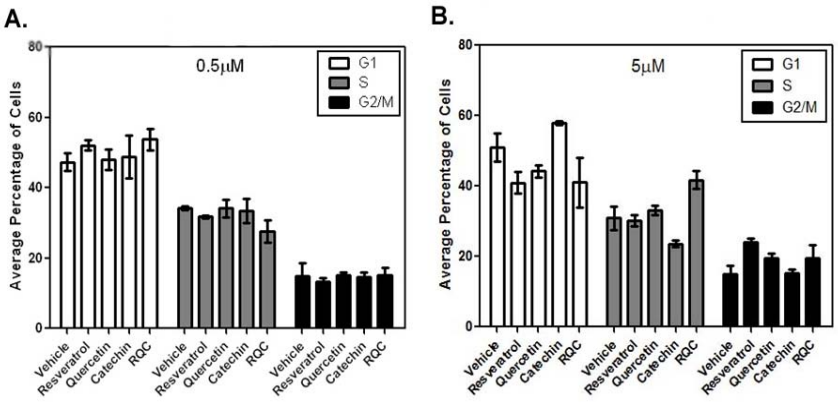


Figure 2. Effect of grape polyphenols on cell cycle progression of MDA-MB-435 cells. MDA-MB-435 cells in 5% serum and phenol red-free media were treated with vehicle, 0.5 or 5μM resveratrol, quercetin, catechin or RQC every 48h for 96h. Flow cytometry analysis of percentage of PI stained cells at each cell cycle phase ± SEM for three individual experiments (n=3) is presented. **A and B,** MDA-BM-435 cells treated with individual or combined resveratrol, quercetin and catechin at 0.5μM (**A**) or 5μM (**B**).

Apoptosis

The effect of RQC treatment on apoptosis of MDA-MB-435 cells was measured by caspase 3 activity and Annexin V staining (Fig. 3). This downstream effector caspase was selected to assess the effect of RQC on both receptor-regulated and mitochondrial apoptotic pathways. Combined grape polyphenols at 0.5μM did not affect caspase 3 activity, while 5μM RQC increased caspase 3 activity by two fold with a $p \leq 0.06$ thus, implicating RQC treatment in apoptosis induction (Fig. 3A). This result was confirmed by Annexin V staining, where 44% of RQC-treated cells were shown to be apoptotic, compared to only 6.8% in vehicle-treated cells (Fig. 3B). Annexin V binds to the phosphatidyl serine residues present on the outer leaflet of the plasma membrane during early apoptosis.

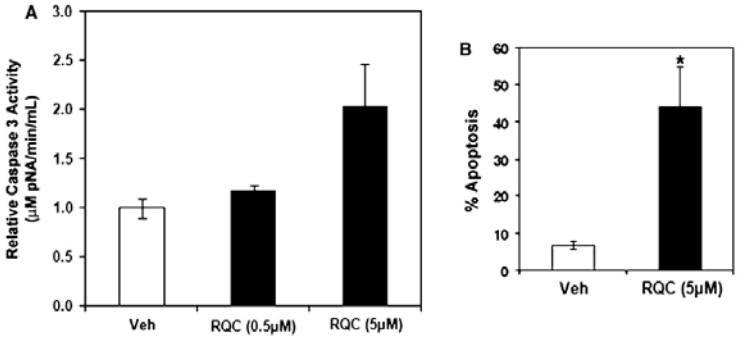


Figure 3. Effect of grape polyphenols on apoptosis of MDA-MB-435 cells. Apoptosis of MDA-MB-435 cells was detected by caspase 3 activity assays (**A**) or fluorescence microscopy for Annexin V staining (**B**) following 48h incubation with vehicle or RQC. **A,** Average caspase 3 activity in mol of pNA/min/mL relative to vehicle (n = 4 ± SEM) as quantified from absorbance at 405nm of the pNA released by caspase 3 activity. **B,** Percentage of cells undergoing apoptosis was calculated by Image J analysis of brightfield (total number of cells) and red fluorescence (apoptotic cells stained with Annexin V-Cy3) from ten random microscopic fields/coverslip. Averages ± SEM are shown for two separate experiments with duplicates for each treatment (n=4). An asterisk indicates statistical significance ($p \leq 0.05$) when compared to vehicle.

The increase in apoptosis induced by RQC suggests that the effect of combined grape polyphenols on cell proliferation might be due not only to cell cycle arrest, but also to induction of apoptosis.

Grape polyphenols inhibit breast cancer cell migration *in vitro:*

The effect of grape polyphenols on MDA-MB-231 and MDA-MB-435 cell migration, a process critical to metastasis, was analyzed *in vitro* by a Transwell Assay. Cells were treated with vehicle, 0.5, or 5μM resveratrol, quercetin, catechin, or RQC in serum-free and phenol red-free media. Following 8 hour incubation, the number of cells that actively migrated to the underside of a membrane was quantified (Fig. 4). In MDA-MB-231 cells, resveratrol and quercetin at 0.5μM increased cell migration in a statistically significant manner (Fig. 4A). The effect of resveratrol is similar to our previous results that reported low concentrations of resveratrol to act comparable to estrogen and increase cell migration [3]. None of the other grape polyphenols significantly changed breast cancer cell migration. At 0.5 and 5μM, combined RQC treatment significantly reduced MDA-MB-231 cell migration by ~60% when compared to vehicle controls (Fig. 4A); whereas, 0.5μM combined RQC treatment reduced MDA-MB-435 cell migration by ~20%, and 5μM RQC significantly reduced cell migration by 40% (Fig. 4B).

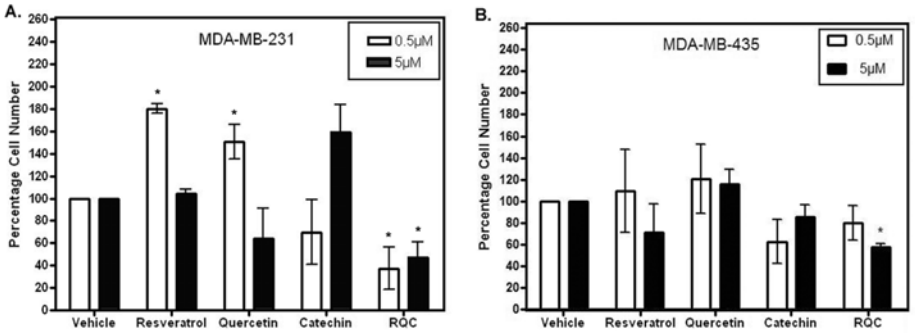


Figure 4. Effect of grape polyphenols on MDA-MB-231 and MDA-MB-435 cell migration. Quiescent MDA-MB-231 (**A**) or MDA-MB-435 (**B**) cells were placed on the top well of Transwell chambers in serum-free, phenol red-free media and the number of cells that migrated through the membrane of the top well in response to various treatments was quantified relative to control. Data (±SEM) are quantified from analysis of 25 microscopic fields/treatment from triplicate treatments. The bottom well contained the following for 8 hours: vehicle, 0.5μM or 5μM resveratrol, quercetin, catechin, or RQC. An asterisk indicates statistical significance ($p \leq 0.05$) when compared to vehicle.

Figures 1 to 4 are published in [4].

Antibody microarrays

MDA-MB-231 cells were treated for 15min with individual or combined resveratrol, quercetin, and catechin at 5μM each for 15min. Total protein extracts were obtained and biotinylated to later be incubated with the arrays. Finally, detection was achieved by incubation of the arrays with streptavidin coupled to the fluorescent dye Cy3. The experiment with the combined polyphenols was conducted three times but unfortunately, reproducibility was not achieved. Nevertheless, western blots were conducted for some of the proteins found to be regulated by RQC after array data analysis (Fig. 5). We were able to confirm that the phosphorylation and therefore, activation of the Ser/Ther protein kinase Akt was reduced by 5μM RQC treatment for 15min not only in the MDA-MB-231 but also in the ER negative and highly metastatic breast cancer cell line MDA-MB-435 (Fig. 6)

	Phospho/Total		
Protein	**Veh**	**RQC**	**FC**
Src (Tyr418)	2.4	1.6	-1.5
Akt2 (Ser474)	2.6	1.8	-1.5
Stathmin 1 (Ser24)	1.6	1.1	-1.5
Stathmin 1 (Ser15)	2.6	1.1	-2.4
CDC2 (Tyr15)	1.9	1.1	-1.6
p44/42 MAP Kinase (Thr202)	1.7	1.1	-1.5
p44/42 MAP Kinase (Tyr204)	2.1	1.4	-1.5
Connexin 43 (Ser367)	2.2	1.2	-1.8
Merlin (Ser518)	1.9	1.2	-1.5

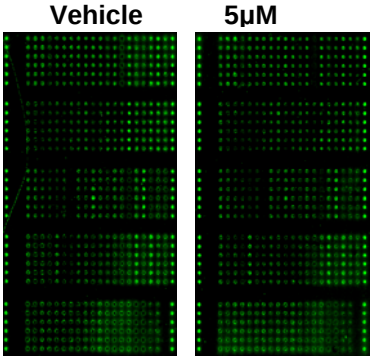
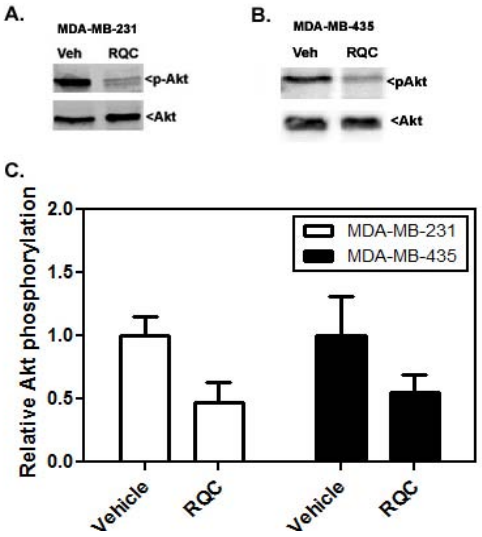


Figure 5. RQC treatment regulates the activity of cytoskeletal signaling proteins on MDA-MB-231 cells. Confluent MDA-MB-231 cells, starved for 24h in serum and phenol red-free media were treated with vehicle or 5μM RQC for 15min. Levels of phospho- and total forms of cytoskeletal signaling proteins were assayed using focused antibody microarrays (FullMoon Biosystems, Inc., CA).



The PI3K/Akt pathway regulates multiple cellular processes important for tumorigenesis such as cell growth, cell proliferation, cell migration, and survival. This pathway is also integral for the epithelial to mesenchymal transition which in cancer cells is indicative of aggressiveness and invasive potential [5,6]. The observed effect of RQC on Akt activity suggests that the possible mechanism by which combined grape polyphenols resveratrol, quercetin and catechin inhibit breast cancer cell proliferation is via S phase arrest and reduced cell survival signaling.

Figure 6. Effect of grape polyphenols on Akt phosphorylation on MDA-MB-231 and MDA-MB-435 cells. Confluent MDA-MB-231 or MDA-MB-435 cells were serum starved for 24h, treated with vehicle or 5μM RQC for 15 min, lysed immediately, and western blotted for total or active (phospho-Akt^{Ser473}) Akt. Representative western blots (from 3 separate experiments) of

MDA-MB-231 (**A**) and (from 2 separate experiments) of MDA-MB-435 (**B**) cell lysates are shown. **C**, Akt activity (phospho-Akt/Akt) relative to vehicle as quantified from Image J analysis of integrated density.

Key Research Accomplishments

We found that grape polyphenols:

- Inhibit cell proliferation, cell cycle progression, and cell migration on ERα(-), ERβ(+) MDA-MB-231 and ER(-) MDA-MB-435 breast cancer cell lines.
- Induce apoptosis in MDA-MB-435 cells.
- Reduce Akt activity by reducing phospho-Akt levels in both MDA-MB-231 and MDA-MB-435 cells.

Reportable Outcomes

A. Publications:

1. **Castillo-Pichardo, L,** Martínez-Montemayor, MM, Martínez, JE, Wall, KM, Cubano, LA, Dharmawardhane, S. Inhibition of mammary tumor growth and metastases to bone and liver by dietary grape polyphenols. Clinical and Experimental Metastasis, 2009, 26(6), 505-516.

B. Poster Presentations:

1. April 2009 – 100th Annual meeting of the American Association for Cancer Research, Denver, CO

Castillo-Pichardo, L, De La Mota-Peynado, A, Martínez-Montemayor, MM, Otero-Franqui, E, Dharmawardhane, S. Inhibitory mechanisms of breast cancer cell and tumor growth by red wine polyphenols**.**

2. December 2008 - 48th Annual meeting of the American Society of Cell Biology, San Francisco, CA

Castillo-Pichardo, L, Dharmawardhane, S. Role of estrogen receptor isoform expression on inhibition of breast cancer progression by grape polyphenols.

3. November 2008 - American Institute for Cancer Research annual research conference on *Food, Nutrition, Physical Activity and Cancer*, Washington, DC

Castillo-Pichardo, L , Martínez-Montemayor, MM, Schlachterman, A, Valle, F, Wall, K., Martínez, JE, De La Mota-Peynado, A, Cubano, L, Dharmawardhane, S. Inhibition of estrogen receptor negative breast cancer progression by grape polyphenols.

Conclusion

The data presented above demonstrates that combined grape polyphenols are more effective than individual compounds at inhibiting biological functions relevant for breast cancer progression, such as cell proliferation, cell cycle progression and cell migration on both estrogen receptor (ER)α(-), ERβ(+) MDA-MB-231 and ER(-) MDA-MB-435 breast cancer cell lines. Akt mediated survival signaling was also reduced by combined grape polyphenols on both cell lines. Additionally, combined grape polyphenols were shown to be effective at induction of apoptosis in MDA-MB-435 cells. However, the effect of combined grape polyphenols was more prominent on MDA-MB-231 than in MDA-MB-435 cells. This difference in response was expected since MDA-MB-435 cells are characterized by having a more aggressive phenotype and are known to be highly metastatic. This finding suggests that grape polyphenols may be acting as antiestrogenic compounds in the MDA-MB-231 cells since this cell line expresses the ERβ isoform and MDA-MB-435 cells are ER negative. Also, since MDA-MB-435 cells are Her2 overexpressing it is possible that combined grape polyphenols are not as efficient at inhibiting the increased Her-2 signaling in this highly aggressive cancer cell line. However, mechanistic studies need to be conducted to further address the differences in response to grape polyphenols between these two cancer cell lines.

References

1. Jemal,A., Murray,T., Ward,E., Samuels,A., Tiwari,R.C., Ghafoor,A., Feuer,E.J. and Thun,M.J. Cancer statistics, 2005, CA Cancer J Clin., *55:* 10-30, 2005.

2. Schlachterman,A., Valle,F., Wall,K.M., Azios,N.G., Castillo,L., Morell,L., Washington,A.V., Cubano,L.A. and Dharmawardhane,S.F. Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model, Transl Oncol, *1:* 19-27, 2008.

3. Azios,N.G. and Dharmawardhane,S.F. Resveratrol and estradiol exert disparate effects on cell migration, cell surface actin structures, and focal adhesion assembly in MDA-MB-231 human breast cancer cells, Neoplasia, *7:* 128-140, 2005.

4. Castillo-Pichardo,L., Martinez-Montemayor,M.M., Martinez,J.E., Wall,K.M., Cubano,L.A. and Dharmawardhane,S. Inhibition of mammary tumor growth and metastases to bone and liver by dietary grape polyphenols, Clin.Exp.Metastasis, 2009.

5. McCubrey,J.A., Sokolosky,M.L., Lehmann,B.D., Taylor,J.R., Navolanic,P.M., Chappell,W.H., Abrams,S.L., Stadelman,K.M., Wong,E.W., Misaghian,N., Horn,S., Basecke,J., Libra,M., Stivala,F., Ligresti,G., Tafuri,A., Milella,M., Zarzycki,M., Dzugaj,A., Chiarini,F., Evangelisti,C., Martelli,A.M., Terrian,D.M., Franklin,R.A. and Steelman,L.S. Alteration of Akt activity increases chemotherapeutic drug and hormonal resistance in breast cancer yet confers an achilles heel by sensitization to targeted therapy, Adv.Enzyme Regul., *48:* 113-135, 2008.

6. Franke,T.F. PI3K/Akt: getting it right matters, Oncogene, *27:* 6473-6488, 2008.

Appendix

Cytoskeletal Phosphorylation Antibody Array

Antibody List

H= human, M = mouse, R = rat

ID	*Antibody Name*	*Reactivity*	*Isotype*	*Source*
1	PDK1(Phospho-Ser241)	HMR	IgG	Rabbit
2	Raf1(Phospho-Ser259)	HMR	IgG	Rabbit
3	MEK-2(Phospho-Thr394)	HMR	IgG	Rabbit
4	Akt(Phospho-Ser473)	HMR	IgG	Rabbit
5	Akt(Phospho-Thr308)	HMR	IgG	Rabbit
6	FAK(Phospho-Tyr861)	HMR	IgG	Rabbit
7	Integrin β3(Phospho-Tyr773)	HM	IgG	Rabbit
8	Ezrin(Phospho-Tyr353)	HM	IgG	Rabbit
9	BAD(Phospho-Ser112)	HM	IgG	Rabbit
10	BAD(Phospho-Ser136)	HM	IgG	Rabbit
11	BAD(Phospho-Ser155)	HM	IgG	Rabbit
12	G3BP-1(Phospho-Ser232)	H	IgG	Rabbit
13	Paxillin(Phospho-Tyr118)	HMR	IgG	Rabbit
14	Caveolin-1(Phospho-Tyr14)	HMR	IgG	Rabbit
15	Src(Phospho-Tyr418)	HMR	IgG	Rabbit
16	cdc25C(Phospho-Ser216)	H	IgG	Rabbit
17	FAK(Phospho-Tyr925)	HMR	IgG	Rabbit
18	Akt2(Phospho-Ser474)	HMR	IgG	Rabbit
19	LIMK1(Phospho-Thr508)	HMR	IgG	Rabbit
20	CDK2(Phospho-Thr160)	HMR	IgG	Rabbit
21	cdc25A (Phospho-Ser75)	HMR	IgG	Rabbit
22	cofilin (Phospho-Ser3)	HMR	IgG	Rabbit
23	Vav (Phospho-Tyr174)	HMR	IgG	Rabbit
24	Src(Phospho-Tyr529)	HMR	IgG	Rabbit
25	VASP(Phospho-Ser238)	HMR	IgG	Rabbit
26	MEK1(Phospho-Ser221)	HMR	IgG	Rabbit
27	Paxillin(Phospho-Tyr31)	H	IgG	Rabbit
28	Ezrin(Phospho-Thr566)	HMR	IgG	Rabbit
29	Raf1(Phospho-Ser338)	HMR	IgG	Rabbit
30	MEK1(Phospho-Ser217)	HMR	IgG	Rabbit
31	VASP(Phospho-Ser157)	HMR	IgG	Rabbit
32	Pyk2(Phospho-Tyr402)	HMR	IgG	Rabbit
33	Stathmin 1(Phospho-Ser24)	HMR	IgG	Rabbit
34	Stathmin 1(Phospho-Ser37)	HMR	IgG	Rabbit
35	Stathmin 1(Phospho-Ser15)	HMR	IgG	Rabbit
36	CDC2(Phospho-Tyr15)	HMR	IgG	Rabbit
37	p44/42 MAP Kinase（Phospho-Thr202)	HMR	IgG	Rabbit
38	p44/42 MAP Kinase(Phospho-Tyr204)	HMR	IgG	Rabbit
39	Connexin 43(Phospho-Ser367)	HMR	IgG	Rabbit
40	Merlin(Phospho-Ser518)	HMR	IgG	Rabbit
41	synapsin(Phospho-Ser9)	HMR	IgG	Rabbit
42	Cortactin(Phospho-Tyr421)	HMR	IgG	Rabbit
43	Cortactin(Phospho-Tyr466)	HMR	IgG	Rabbit
44	Integrin β3(Phospho-Tyr785)	HM	IgG	Rabbit

45	MEK1(Phospho-Thr291)	HMR	IgG	Rabbit
46	PKCδ (Phospho-Ser645)	HMR	IgG	Rabbit
47	PKCθ(Phospho-Ser676)	HMR	IgG	Rabbit
48	PDK1(Ab-241)	HMR	IgG	Rabbit
49	Raf1(Ab-259)	HMR	IgG	Rabbit
50	MEK-2(Ab-394)	HM	IgG	Rabbit
51	Akt(Ab-473)	HMR	IgG	Rabbit
52	Akt(Ab-308)	HMR	IgG	Rabbit
53	BAD(Ab-112)	HM	IgG	Rabbit
54	BAD(Ab-136)	HMR	IgG	Rabbit
55	BAD(Ab-155)	HMR	IgG	Rabbit
56	FAK(Ab-861)	HMR	IgG	Rabbit
57	Integrin β3(Ab-773)	HM	IgG	Rabbit
58	Ezrin(Ab-353)	HM	IgG	Rabbit
59	G3BP-1(Ab-232)	H	IgG	Rabbit
60	Paxillin(Ab-118)	HMR	IgG	Rabbit
61	CDK2(Ab-160)	HMR	IgG	Rabbit
62	Caveolin-1(Ab-14)	HMR	IgG	Rabbit
63	Src(Ab-418)	HMR	IgG	Rabbit
64	LIMK1(Ab-508)	HMR	IgG	Rabbit
65	cdc25C(Ab-216)	H	IgG	Rabbit
66	FAK(Ab-925)	HMR	IgG	Rabbit
67	Akt2(Ab-474)	HMR	IgG	Rabbit
68	cdc25A (Ab-75)	HMR	IgG	Rabbit
69	cofilin (Ab-3)	HMR	IgG	Rabbit
70	Vav (Ab-174)	HMR	IgG	Rabbit
71	Src(Ab-529)	HMR	IgG	Rabbit
72	VASP(Ab-238)	HMR	IgG	Rabbit
73	MEK1(Ab-221)	HMR	IgG	Rabbit
74	Paxillin(Ab-31)	H	IgG	Rabbit
75	Ezrin(Ab-566)	HMR	IgG	Rabbit
76	Raf1(Ab-338)	HMR	IgG	Rabbit
77	MEK1(Ab-217)	HMR	IgG	Rabbit
78	VASP(Ab-157)	HMR	IgG	Rabbit
79	FAK(Ab-397)	HMR	IgG	Rabbit
80	Pyk2(Ab-402)	HMR	IgG	Rabbit
81	Stathmin 1(Ab-24)	HMR	IgG	Rabbit
82	Stathmin 1(Ab-37)	HMR	IgG	Rabbit
83	Stathmin 1(Ab-15)	HMR	IgG	Rabbit
84	CDC2(Ab-15)	HMR	IgG	Rabbit
85	p44/42 MAP Kinase（Ab-202)	HMR	IgG	Rabbit
86	p44/42 MAP Kinase(Ab-204)	HMR	IgG	Rabbit
87	Connexin 43(Ab-367)	HMR	IgG	Rabbit
88	Merlin(Ab-518)	HMR	IgG	Rabbit
89	synapsin(Ab-9)	HMR	IgG	Rabbit
90	Cortactin(Ab-421)	HMR	IgG	Rabbit
91	Cortactin(Ab-466)	HMR	IgG	Rabbit
92	Integrin β3(Ab-785)	HM	IgG	Rabbit
93	MEK1(Ab-291)	HMR	IgG	Rabbit
94	PKCδ (Ab-645)	HMR	IgG	Rabbit
95	PKCθ(Ab-676)	HMR	IgG	Rabbit