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Review

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Influence of Berry Polyphenols on Receptor Signaling and Cell-Death Pathways: Implications for Breast Cancer Prevention

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ABSTRACT: Breast cancer is the most commonly diagnosed cancer among women worldwide. Many women have become more aware of the benefits of increasing fruit consumption, as part of a healthy lifestyle, for the prevention of cancer. The mechanisms by which fruits, including berries, prevent breast cancer can be partially explained by exploring their interactions with pathways known to influence cell proliferation and evasion of cell-death. Two receptor pathways, estrogen receptor (ER) and tyrosine kinase receptors, especially the epidermal growth factor receptor (EGFR) family, are drivers of cell proliferation and play a significant role in the development of both primary and recurrent breast cancer. There is strong evidence to show that several phytochemicals present in berries such as cyanidin, delphinidin, quercetin, kaempferol, ellagic acid, resveratrol, and pterostilbene interact with and alter the effects of these pathways. Furthermore, they also induce cell death (apoptosis and autophagy) via their influence on kinase signaling. This review summarizes in vitro data regarding the interaction of berry polyphenols with the specific receptors and the mechanisms by which they induce cell death. This paper also presents in vivo data of primary breast cancer prevention by individual compounds and whole berries. Finally, a possible role for berries and berry compounds in the prevention of breast cancer and a perspective on the areas that require further research are presented.

KEYWORDS: *berries, berry polyphenols, breast cancer, ellagic acid, cyanidin, delphinidin, quercetin, kaempferol, resveratrol, estrogen receptor, epidermal growth factor receptor, kinase signaling, apoptosis, autophagy, ACI rats*

INTRODUCTION

Cancer development and metastasis is a multistep process and a result of the dysfunction of several regulatory features that keep the cells in check.[1] Although food has not been posited as a cure for cancer, several lines of evidence exist supporting the belief that components of food can affect the development of cancer in both beneficial and detrimental ways.[2,3] The reason is the intense interaction of the food components with the cells at different stages during cancer development. Breast cancer development follows this rule as well. Healthy changes in lifestyle, including a better diet, may prevent up to 40% of breast cancers.[4] Increasing the consumption of fruits and vegetables is one part of a healthy dietary modification. Berries contain phytochemicals that have been shown to interfere, in a beneficial way, with multiple pathways linked to the development of cancer. These compounds are bioavailable and can potentiate each others' effects. In addition, they taste good, are a part of the Western culinary repertoire, are grown locally, and are available year-round as fresh or frozen varieties. They are also a constant media presence, leading to increased awareness regarding their effects among consumers. A recent study reports that there is a high correlation between increased fruit and vegetable consumption in cancer survivors that actively seek health information in the media.[5] Thus, a general case can be made for the study of berries, as fruits, in the prevention cancer. A particular case can be made for breast cancer because women form a large part of the media consumers and are more likely to change their dietary behavior on the basis of such information. One purpose of this review is to present to the scientific community the evidence that is available regarding the benefits of berries for breast cancer prevention.

The health benefits of berries have been linked mostly to their antioxidant effects. Although this is an important contributor, berry phytochemicals also interact with other pathways, especially receptor signaling. In this review, we focus on two receptor signaling pathways known to play key roles in breast cancer development, estrogen receptor (ER) and tyrosine kinase receptor (TKR). These two pathways are important for bestowing proliferative potential to breast cancer cells and allowing the evasion of cell death. Berry phytochemicals can interfere with both ER and TKR signaling. They can also induce apoptotic and/or autophagic cell death by modulating kinase signaling, which is also involved in the cross-talk between ER and TKR pathways. We evaluate the in vitro data available in the literature for these modulations and apply it to breast cancer prevention. The mechanism by which well-studied compounds such as resveratrol and quercetin induce cell death in breast cancer cell lines is available, but there is a lack of such information for compounds such as cyanidin, delphinidin, and pterostilbene. Because there is a generality among various cell lines in how a phytochemical induces cell death, we present mechanistic data generated in other cell lines

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for the latter. However, we emphasize the importance of confirming these in breast cancer specific cell lines. We also present in vivo data available from existing animal models of breast cancer. Finally, we provide a perspective on how whole berries and berry compounds can be used for the prevention of primary and recurrent breast cancer and the areas of research that need to be explored further.

ESTIMATED INTAKE AND PLASMA LEVELS OF BERRY POLYPHENOLS

We begin by discussing the berries that are commonly consumed, the different classes of polyphenols present in them, and the typical plasma concentrations of these phytochemicals. It is useful to understand the physiological levels that can be achieved by oral administration to contrast it with those in in vitro studies that tend to use supraphysiological doses. The berries most commonly consumed in the American diet are blackberry, blueberry, cranberry, raspberry (including black raspberry), and strawberry. Although they are good sources of micronutrients such as vitamin C and selenium, they are richer sources of polyphenols.[6] Berries vary greatly in their chemical composition, which is affected by agricultural and geographical variations as well as species. Instead of focusing on a single type of berry or a single polyphenol, this review will evaluate several polyphenols typically found in the most commonly consumed berries. We will consider five classes of polyphenols, anthocyanins, flavonols, tannins, stilbenes, and lignans, predominantly found in berries (Figure 1). Of these,

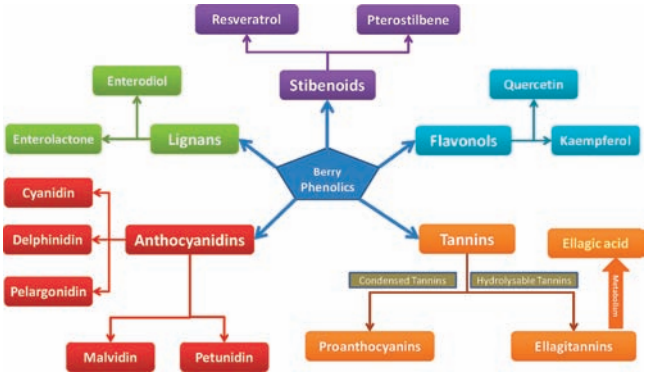

Figure 1. Classification of berry phenolics and representative polyphenols from each class.

anthocyanins are the most ubiquitous and the primary flavonoids responsible for the vibrant colors of berries.[7] Table 1 lists the various polyphenols often found in commonly consumed berries, their primary berry sources, and estimated levels of consumption in the U.S. population.

The relative abundance of various polyphenols present in berries is of the order anthocyanins > proanthocyanins > ellagitannins > flavonols > lignans > stilbenes (Table 1). However, the relative intake in the U.S. population is proanthocyanins > flavonols > anthocyanins > ellagitannin > stilbenes/lignans (Table 1). This is reflective of the contributions from other food sources to the intake. Although proanthocyanins are consumed at a higher level, they are less bioavailable than anthocyanins. This is reflected in the peak plasma levels seen for anthocyanidins (115 nM) versus proanthocyanidins (40 nM). The average intake of anthocyanins is 12.5 mg/person/day, whereas that of proanthocyanin is 57 mg/person/day.[8] Due to limited bioavailability and extensive clearance/metabolism in the body, the typical plasma concentrations of these compounds occur in the nanomolar range. Nevertheless, low micromolar ranges have been reported for resveratrol, quercetin, and enterolactone (Table 1). These micromolar levels were achieved by feeding foods other than berries. Both the gut (gastric, intestinal, and colonic) and the liver metabolites of these compounds play a significant role in mediating the physiological effects of these compounds. The important role of metabolites is briefly discussed later. It must be kept in mind that although individual compounds may affect a pathway one way, the presence of several polyphenols together may change these effects. In this review, we have chosen to focus on a select group of polyphenols and their metabolites when applicable, on the basis of either the abundance of the polyphenol in berries (ellagic acid, anthocyanidins) or the data available for their interaction with the cell-signaling pathways mentioned (resveratrol, quercetin, and lignans).

RECEPTOR PATHWAYS THAT PLAY A KEY ROLE IN BREAST CANCER DEVELOPMENT

The development of primary and secondary neoplasia is a highly complex process involving multiple pathways and bidirectional cross-talk among these pathways. In this section, we discuss the alterations in two cell-signaling pathways known to play a role in both primary cancer and acquired antiestrogen (AE)-resistant secondary cancer. These are estrogen receptor (ER) and tyrosine kinase receptor (TKR) pathways. Kinase signaling, the key connector of these pathways, is also discussed. The effect of individual berry phytochemicals, berry extracts, and whole berries on cancer cells mediated via these pathways is summarized. The mechanisms by which molecules of these pathways can influence cancer development include but are not limited to alterations in expression, activity, regulation by upstream and downstream molecules, phosphorylation, and epigenetic modifications.

Role of Estrogen Receptor (ER) Signaling in Breast Cancer Cells. Estrogen receptors (ER) are central to the development of the normal mammary gland, as well as primary and secondary breast cancers. ER action in the breast epithelium can be classified into two: nuclear initiated steroid signaling (NISS), classic action via estrogen response elements (ERE) and nonclassic action via AP-1, SP1, and other transcription factors; and membrane-initiated steroid signaling (MISS) (Figure 2).[9,10] The complexity of how ER expression and signaling affects carcinogenesis is summarized in Table 2 and reviewed in ref 11. In the normal mammary gland ER-positive cells are fewer and do not proliferate; instead, they produce paracrine growth factors that signal proliferation in adjacent cells (reviewed in refs 12 and 13). During primary ER-positive neoplasia, many proliferating cells are ER+ and possibly convert paracrine to autocrine growth signaling. Over 70% of breast tumors diagnosed are ER+.[14] ER+ status is a key diagnostic criterion for the choice of AE or aromatase inhibitor (AI) treatment in patients. Many AE-treated tumors retain their ER expression during recurrence, but signaling through the ER pathway is altered in these resistant tumors.[10,14] Thus, it is clear that targeting this pathway using berry polyphenols may affect the development of both primary and secondary cancers.

Effect of Berry Polyphenols on ER Signaling. Several berry phytochemicals interact with the ER, and Table 3

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Table 1. Intakes and Plasma Concentrations of Select Polyphenols Found in Commonly Consumed Berries

polyphenol class	berry polyphenol	berry source[a]	quantity present (mg/kg fresh weight)[b]	estimated intake (mg/person/day)[c]	plasma level[d]	other food source	refs
anthocyanidins	cyanidin	blackberry	417–6870	12.5	1.4–115 nM	red cabbage,	8, 119
	delphinidin	black raspberry				grapes	
	malvidin	blueberry				pomegranate	
	pelargonidin	cranberry				red wine	
	petunidin	raspberry					
		strawberry					
flavonols	quercetin	cranberry	6–263 (total)	25–35	0.08–7.6 μM	apples, onions	90, 119
	kaempferol	strawberry	quercetin (6–121) ≫ kaempferol (5–8)				
ellagitannins	ellagic acid	blackberry	**57–537**	1	60–100 nM	pomegranate	120–123
		black raspberry				walnuts	
	urolithins (ellagic acid metabolites)	raspberry				oak-aged whiskey	
		strawberry					
proanthocyanidins	procyanidin	blackberry	302–4188	57.7	40 nM	chocolate	121, 124, 125
	prodelphinidin	blueberry				apples	
	propelargonidin	cranberry				grapes	
		strawberry				tea	
phytoalexins–stilbenes	resveratrol	blueberry	**0.7–85 μg/kg**	0.9[e]	2–3.3 μM[f]	wine	126–129
		cranberry				grapes	
						peanuts	
	pterostilbene	blueberry	**9.9–15.1 μg/kg**			grapes	
lignans	secoisolarisinol	blackberry	13.9–371	0.6	1.7 μM (enterolactone)	flax seed	82, 130
		blueberry				rye bread	
		raspberry				sesame seed	
		strawberry					

[a]Berries with the highest reported content of the selected polyphenols. Neither polyphenols nor berries are arranged in order of specific contents. [b]Some quantities (in bold) have been converted from dry weight values using the formula 1 g dry wt = 10 g fresh weight for most berries. [c]All estimated intakes are for US population unless specifically stated. [d]Plasma levels are collected from a wide variety of human intervention studies. They are representative of peak plasma levels and are not representative of U.S. population. [e]Estimated intake for Spanish population cohort from the EPIC study.[129] [f]Calculated from raw value 2.7 mg/3.6 L plasma using formula μM = μg/L ÷ molecular weight of resveratrol (228.4).[126]

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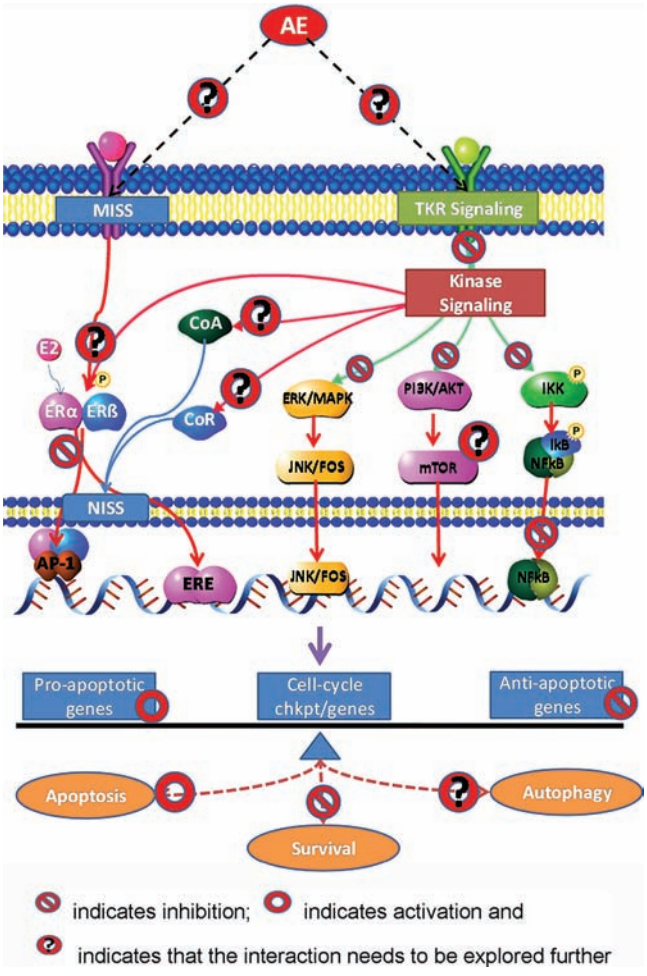

Figure 2. Simplified schematic of the influence of berry polyphenols on cell-signaling pathways in breast cancer.

Table 2. Effect of Estrogen Receptor Signaling on Breast Carcinogenesis

ER signaling component	mechanism by which it affects carcinogenesis	effect of berry polyphenols	refs
type of ER (α or β)	expression pattern in tumors ERα/ERβ ratio	some bind more avidly to ERβ than to α (Table 3)	11, 131, 132
mutant forms of ER	altered ligand and DNA binding	NR[a]	11, 133
coactivators	increased expression	NR	134, 135
corepressors	decreased expression	NR	29, 136
phosphorylation	can cause constitutive activation of ER	NR	

[a]NR, not reported in the literature to the best of our knowledge.

summarizes these interactions. It is currently accepted that ERα plays a pro-proliferative role and is involved in growth-promoting actions, whereas ERβ may counteract its effects.[15−17] Most dietary polyphenols bind to these receptors with 100−1000-fold weaker affinity than estrogen.[18−20] Studies presented in Table 3 were performed using either direct ligand binding or ERE-reporter assays, which measure the ability of phytochemicals to bind to either ERα or ERβ and cause downstream effects. Polyphenols do not bind equally avidly to both receptors and often show preference for one or the other. In ligand binding assays, quercetin and kaempferol have a higher affinity for ERβ,[21−23] whereas resveratrol and enterolactone have a higher affinity for ERα.[24,25] In breast cancer cells, anthocyanidins, cyanidin, delphinidin, and pelargonidin, bind to ERs and significantly reduce 17β estradiol (E2)-induced ERE-luciferase expression, acting like an antiestrogen.[26] However, whether they preferentially bind to ERα or ERβ is not reported. In HeLa cells transfected with either ERα or ERβ, ellagic acid acts like an estrogen in ERα-transfected cells, but like an antiestrogen in ERβ cells.[27] In MCF-7 cells, metabolites of ellagic acid, urolithins A and B, bind avidly to ERα and ERβ, respectively.[28] This suggests that the effect of ellagic acid on the tissue may be dependent on the type of ER primarily expressed and also on the metabolite generated. Many of these phytochemicals are agonists of ER, in that they can induce cell proliferation in MCF-7 cells in the absence of E2. However, in the presence of E2, they antagonize E2 action, thus acting as antiestrogens.[28] In such in vitro studies, the dose used may have a specific effect on the outcome. For example, if a particular polyphenol is used at a very high concentration in culture, it could simply compete for the receptor and displace E2, the natural ligand, resulting in an antiestrogenic activity. Quercetin, kaempferol, and resveratrol show distinct dose-dependent biphasic effects (Table 3). It has been postulated that because these compounds bind to ERβ with a greater affinity than to ERα their growth-retarding effects are ERβ-mediated.[21,24] However, quercetin stimulates MCF7 cell proliferation at 10 μM, whereas it inhibits it significantly at 100 μM,[22] supporting our theory of dose-dependent displacement of the natural ligand.

There is evidence to suggest than many of these phytochemicals act as selective estrogen receptor modulators (SERM) similar to Tamoxifen (TAM) and its active metabolite 4-hydroxytamoxifen (4-OHT). TAM/4-OHT can act as an agonist in the absence of E2 and as an antagonist in its presence.[14] Furthermore, similar to 4-OHT, these compounds bind to ERβ with a higher affinity than to ERα.[29] In addition, several berry phytochemicals can act as pure agonists in cell types other than mammary epithelial cells.[27,30,31] Many studies have blocked ER activity using either 4-OHT or ICI 182,780 (ICI; an ER-degrading AE) to show that the effect of these phytochemicals is ER-mediated.[26,27,32] However, data on whether these compounds antagonize, synergize, or potentiate the effects of AE in breast cancer cells are very limited. Our preliminary data show that ellagitannin−punicalagin and ellagic acid cause a synergistic cytotoxicity in combination with subtoxic levels of 4-OHT and ICI in MCF-7 cells (Woode and Aiyer, unpublished data). We know of no such information for other berry compounds or their respective metabolites.

Coregulators are molecules that either potentiate (coactivator) or repress (corepressors) the transcriptional activity of the steroid receptors. Very few data are available on whether polyphenols modulate the selective recruitment of coregulators to ERα or ERβ. It can be inferred from Klinge et al.[33] that the estrogenic action of resveratrol requires coregulator recruitment because resveratrol fails to elicit a response in a yeast expression system but shows a dose-dependent activation of ERβ in mammalian cells. Indeed, resveratrol does differentially recruit ER coactivators SRC-1 and GRIP-1 to either ERα or ERβ depending on the dose used. At a dose of 1−10 μM, it preferentially recruits SRC-1 to ERβ.[24] This effect is not seen at 100 μM, at which dose it does not recruit either coactivator to

Table 3. Interaction of Berry Polyphenols with ER Signaling[a]

polyphenol	ER binding	assay type	effect	dose	cell type	expression changes	refs
cyanidin	Y	ligand binding; ERE-luciferase	antiestrogenic		MCF-7	↓ ERβ expression	26, 137
delphinidin	Y		antiestogenic			↓ ERα expression	
pelargonidin	Y		antiestrogenic				
quercetin	Y (ERβ ≫ ERα)		estrogenic/antiestrogenic	biphasic effects	MCF-7 MDA-MB-231	↑ ERα and ERβ mRNA	21, 22, 138
kaempferol	Y (ERβ ≫ ERα)	ligand binding; yeast transactivation	estrogenic/antiestrogenic	biphasic effects	MCF-7	↓ ERα mRNA and protein ↓ PGR, cyclin D1 and IRS-1	21, 23, 139
resveratrol	Y (ERα > ERβ)	ERE-luciferase	estrogenic/antiestrogenic	biphasic effects	Ishikawa cells with stable ERs; MCF-7	↓ ERα by proteasomal degradation ↓ cathepsin D and pS-2 exhibits biphasic effects on EGFR-ER cross talk via dose-dependent induction of AKT	24, 140
ellagic acid	Y	ERE-luciferase	estrogenic (via ERα)/ antiestrogenic (via ERβ)		HeLa	↑ IGFBP3 levels similar to ICI 182,780 co-treatment with ICI abrogates EA effect	27
urolithin A[b]	Y (ERα ≫ ERβ)	ligand binding assay	antiestrogenic		MCF-7		28
urolithin B[b]	Y (ERβ ≫ ERα)		antiestrogenic				
enterolactone[b]	Y (ERα ≫ ERβ)	ERE-luciferase	partial agonist		Ishikawa cells with stable ERs; MCF7; HeLa	↑ ERβ ↓ E2-induced VEGF	24, 25, 85

[a]Cell line origins: breast, MCF-7 (ER+), MDA-MB-231 (ER−); cervical, HeLa (ER−); endometrial, Ishikawa (ER−). ER, estrogen receptor; ERE, estrogen receptor response elements; PGR, progesterone receptor; IRS1, insulin receptor substrate 1; IGFBP3, insulin like growth factor binding protein-3; VEGF, vascular endothelial growth factors. [b]Urolithins A and B are gut metabolites of ellagic acid, and enterolactone is a gut metabolite of lignans.

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Table 4. Berry Polyphenol Interaction with Growth Factor Receptor Signaling and Its Downstream Effects[a]

polyphenol	GFR type	cell line	dose	effect observed	refs
cyanidin	EGFR	A431 (human vulva carcinoma)	0.8 μM	↓ tyrosine kinase activity ↓ phosphorylation of ELK-1 and MAPK-1	38
	HER2	MDA-MB231 BT474 MCF-7^{ErbB2} (ethanol mediated)	10−40 μM	↓ cell migration ↓ autophosphorylation ↓ phosphorylation of FAK and p130^{cas} ↓ association of FAK and p130^{cas} to ErbB2	141
delphinidin	EGFR	AU565 MCF-10 A	40 μM	↓ autophosphorylation ↓ phosphorylation of AKT, ERK1/2, JNK1/2/P38	142
		A431	1.3 μM	↓ tyrosine kinase activity	38
	HER2	SKBR3	12.5−100 μM	↓ autophosphorylation ↓ phosporylation of ERK1/2 ≫ AKT	41
quercetin	EGFR	HT29 (colon carcinoma)	0.5−10 μM	↓ autophosphorylation ↓ phosphorylation of ERK1/2	143
	HER2	SKBR3	100−200 μM	↓ tyrosine kinase activity ↑ ubiquitination	144
		MCF-7		↓ expression ↓ phosphorylation of PI3K and AKT	
ellagic acid	EGFR	HT29	65 nM	↓ tyrosine kinase activity ↔ autophosphorylation	37
			100 μM	↓ cell growth	
resveratrol	EGFR	MDA-MB-231	50 μM	↓ phosphorylation of FAK ↓ cell migration and invasion	145
	HER2	MCF-7 (heregulin B1 induced)	2−10 μM	↔ autophosphorylation ↓ phosphorylation of ERK1/2 ↓ ERK-mediated MMP9 activation	146
pterostilbene	HER2/3	MCF-7 (heregulin B1 induced)	5−20 μM	↓ phosphorylation of AKT and p38 ↓ MMP-9 ↔ phosphorylation of ERK1/2	147
procyanidins	EGFR	HT29	5−50 μM	↓ tyrosine kinase activity ↓ autpphosphorylation	148

[a]Abbreviations: ELK-1, E 26 (ETS)-like transcription factor 1; MAPK-1, mitogen activated protein kinase 1; FAK, focal adhesion kinase; p130^{cas}, Crk-associated protein; ERK, extracellular signal regulated kinase; JNK, c-Jun N-terminal kinase; PI3K, phosphatydylinositol-3-phosphate kinase; MMP, matrix metalloproteinase.

ERα or ERβ. At this dose, it simply acts as an ER antagonist. Such results underscore the importance of using physiological concentrations in culture to glean mechanistic insights. Regardless, the effect of berry polyphenols on differential recruitment of coregulators to the ER is an area that still needs much research.

The effect of berry polyphenols on ER signaling is a sum of several mechanisms such as direct interaction with receptor, specificity for receptor isoform (α or β), competition with ligand for receptor binding, and differential recruitment of coregulators. The mechanism likely varies depending on the cell type, dose, and the polyphenol involved. Given that pharmaceutical agents used for endocrine therapy of breast cancer such as TAM and ICI often utilize similar mechanisms, it is important to study the drug−nutrient interaction and how this may affect the outcome of treatment.

ROLE OF GROWTH FACTOR RECEPTOR (GFR) SIGNALING IN BREAST CANCER CELLS

Membrane growth factors are activated by extracellular ligands and activate targets through phosphorylation of upstream kinases, which then leads to activation of downstream transcription factors. One of their functions during normal development is to stimulate cell proliferation in response to external growth factors.[12] In breast cancer, GFRs become overactivated via gene amplification, chromosome translocation, and mutations, leading to a constant stimulation of cell proliferation. Although many members of GFR signaling affect mammary tumorigenesis, the epidermal growth factor receptor (EGFR) family in particular is discussed here. The EGFR family consists of EGFR (ErbB1), HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4, and the activation of downstream targets depends on the dimerization of any two members of the family in response to ligand binding. Expression of EGFR is increased in 30−60% of triple-negative (ER-, PR-, and HER2-negative) breast cancers.[12] Growth factor signaling induces the nongenomic activation of the ER pathway by phosphorylation

Table 5. Control of Kinase Signaling by Berry Polyphenols and Effects on Cell Cycle, Apoptosis, and Autophagy[a]

polyphenol	cell line/dose	cell cycle arrest	cell death pathway	markers	proposed mechanisms	kinase pathway	refs
cyanidin	HS578T/10 μM	G2/M	apoptosis	↓ CDK1, CDK2, cyclin B, cyclin D PARP cleavage	caspase-dependent		149
delphinidin	SMMC7721/150 μM		autophagy	↑ LC3 II inhibited by 3MA and bafilomysin no ↑ in caspase activity	no ER stress	class III PI3K	58
	AU565/40 μM		apoptosis	↑ PARP cleavage ↓ BCL2 ↑ BAX	inhibition of EGFR signaling	PI3K/AKT MAPK	142
	Hep G2/100 μM		apoptosis	↑ caspase 3, PARP cleavage ↓ BCL2 ↑ BAX	oxidative stress mediated	JNK ↑ cJun mRNA; ↑ p-JNK	54
	PC3/180 μM	G2/M	apoptosis	↑ caspases, PARP cleavage, BAX, p21, p27 ↓ BCL2	inhibition of NFκB signaling	IKKΓ (↓ p-IKK)	150
quercetin	MCF-7/150 μM	S	apoptosis	↓ BCL2, cyclin A, B, procaspase 8,12 and CDK2 ↑ PERK, GRP78, CHOP/GADD153, p53 and p57 ↓ mitochondrial membrane potential ↑ caspases 6,8,9	ER stress and UPR ↑ Ca^{2+} influx, ↔ ROS		151
	MCF-10A, MDA-MB-231/100 μM	G1	apoptosis	↑ p-p53, p21 ↓ BCL-xl, cyclin B1 ↔ PTEN, cyclin D, p27, cdc2, and p-cdc2	P53 dependent		152
	AGS, MKN28		autophagy	↑ LC3 II, AVO	beclin-1 mediated, HIF1-α	AKT/mTOR	153
kaempferol	MCF-7, MDA-MB-231/30 μM	G1	apoptosis	↑ PARP cleavage ↑ MEK1 and p-ELK1	ROS mediated	ERK1/2 (sustained activation)	55
ellagic acid (urolithins)	Caco-2/10 μM	G1	apoptosis	↓ cyclins A and B, BCL-xl ↑ cyclin E	Fas-dependent caspase 8-independent, mitochondrial membrane mediated	ERK1/2 (↑ activation)	154, 155
resveratrol	MDA-MB-231/50 μM		apoptosis	↓ BCL2 ↔ BAX, BAD, BCL-xl JNK, and p38 inhibition	MEK1/2 mediated	ERK1/2 (sustained activation)	56
	MCF-7/150 μM		apoptosis	↓ BCL2 ↔ caspase 8 activity ↔ PARP cleavage ↓ $\Delta\psi_m$	ROS mediated NFκB dependent	PI3K	140
	MCF-7vc and MCF-7^{casp3}/64 μM		autophagy/ apoptosis	cell death in MCF-7^{vc} ≫ MCF-7^{Cap3} caspase expression ↓ autophagy LC3 II and GFP-LC3 not ↓ by BECN1 or hvP534 knockdown	noncanonical BECN1-independent atg7-dependent	AKT/mTOR (↓ p-AKT; inhibition of mTOR)	156
pterostilbene	T24 and T24R/100 μM	S (50−75 μM) G1 (100 μM)	apoptosis/ autophagy	↓ cyclins A, B, D1, p-Rb ↓ caspase 3 activity ↑ LC3 II, AVO ↑ BCL2, BCL-xl ↔ BAX, BAD	BECN1-dependent (3MA and BECN1 shRNA inhibit action)	AKT/mTOR (↓ p-AKT/mTOR) ERK1/2 activation	59

[a]Cell line origins: breast, AU565, HS578T, MCF-10A, MCF-7, MDA-MB-231, MCF-7^{vc}, MCF-7^{casp8}; liver, SMMC7721, HepG2; gastric, AGS, MKN28; prostate, PC3; bladder, T24, T24R; colon, Caco-2. Abbreviations: CDK, cyclin dependent kinase; PARP, poly(ADP-ribose) polymerase; BCL2, B-cell lymphoma 2; BAX, Bcl-2 associated X protein; BAD, Bcl-2 associated death promoter; PERK, protein kinase RNA-like endoplasmic reticulum kinase; GRP78, glucose regulated protein 78 (also called BiP or HSPA5); CHOP, ccaat enhancer binding protein homologous protein; PTEN, phosphatase and tensin homologue; cdc, cell division cycle; LC3, microtubule associated protein light chain 3; AVO, autophagic vacuoles; ELK, E26 (ETS)-like transcription factor 1.

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of both the ER and its cofactors.[34,35] This cross-talk between GFR and ER pathways, facilitated via the kinase signaling cascade, is thought to play an important role in conferring TAM resistance. Inhibition of this cross-talk using the EGFR inhibitor Gefitinib abolishes the stimulatory effect of TAM on HER2-overexpressing cells.[36]

EFFECT OF BERRY POLYPHENOLS ON GFR SIGNALING

Berry polyphenols inhibit the tyrosine kinase activity of EGFR and HER2, leading to reduced autophosphorylation, a key step in receptor activation (Table 4). The inhibition of this step leads to reduced phosphorylation of several kinases such as phosphotidylinositol-3-phosphate kinase/protein kinase B (PI3K/AKT) and extracellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK). Table 4 summarizes the effect of various berry polyphenols on EGFR, HER2, and their downstream targets. Polyphenols can interfere with TKR action by two possible mechanisms: inhibiting tyrosine kinase activity and preventing the dimerization between the various EGFR family members. There is clear evidence that many berry polyphenols inhibit the tyrosine kinase activity of EGFR and HER2 purified from the cell membrane. This also occurs in intact cells, albeit at considerably higher doses (Table 4).[37,38] However, evidence of whether these polyphenols inhibit the dimerization of GFRs is not known. In a well-designed study, Weinstein and co-workers showed that the green tea polyphenol epigallocatechin gallate (EGCG) and extract polyphenon E can inhibit the EGF-induced dimerization of EGFR in HT29 cells by disruption of lipid order in the plasma membrane.[39] They also found that cyanidin and delphinidin (50 μM) do not cause disruption of lipid order, but their effect on EGFR dimerization was not tested. The authors report exposure times of 5−30 min. Thus, it is not clear whether plasma membrane incorporation of EGCG plays any role in the observed effects. In a separate study, it has been shown that elderberry anthocyanins are incorporated into the plasma membrane of endothelial cells after 4 h.[40] It would be interesting to explore whether berry polyphenols inhibit the dimerization and subsequent activation of the TKR either by their ability to incorporate into the cell membrane or by direct binding to the receptors.

Many studies presented in Table 4 have been conducted in cell lines that overexpress EGFR or HER2 with or without ER. However, the cross-talk between EGFR activation and ER phosphorylation has rarely been explored in these. With respect to TAM cotreatment, it is unknown whether any of the selected berry compounds selectively potentiate or inhibit its effects and how this may affect the development of TAM resistance. Cotreatment of BT474 or SKBR cells with delphinidin (6−24 μg/mL) significantly reduces the effect of HER2 inhibitors Herceptin and Lapatinib.[41] Although the authors report significant inhibition of HER2 signaling in these cells, they do not mention whether delphinidin actually binds to the HER2 receptor. Therefore, it can only be speculated that the reduced efficacy of the drugs is due to the binding of HER2 receptor by delphinidin, but the exact mechanism is unknown.

It is clear that many berry polyphenols can inhibit the tyrosine kinase signaling component of the EGFR family of TKRs. Constitutive activation of TKR and the subsequent activation of ER through phosphorylation are involved in the development of TAM-resistant recurrent tumors.[42] However, very little is known about whether the presence of one or more polyphenols can inhibit the development of TAM resistance and whether their effect on the EGFR/HER2 pathway plays a significant role in this. More research is warranted to understand the mechanisms by which berry polyphenols affect the EGFR/HER2 pathway and its cross-talk with ER signaling, both in the presence and in the absence of TAM.

EVASION OF CELL DEATH MECHANISMS IN BREAST CANCER

Programmed cell death in cancer cells can be classified into at least three types: apoptosis, autophagy, and necroptosis.[43,44] They play important and defined roles in the normal development during ductal elongation, alveolar development, and especially the postlactional involution of the mammary gland.[45,46] The balance between survival and death in breast epithelial cells is determined by induction of one or more of these mechanisms by various signaling molecules (Figure 2). The two mechanisms pertaining to breast cancer development, apoptosis and autophagy, are discussed herein. Whereas apoptosis has been studied extensively, autophagy is a relatively new field; its possible correlation is being explored further. In primary breast cancer, cancer cells evade apoptosis to survive. Resistance to apoptosis caused by increased expression of anti-apoptotic and/or reduced expression of pro-apoptotic molecules is often seen in breast cancer. Induction of apoptosis by a drug leads to reduction in cell survival and tumor growth.[47,48] Autophagy is a mechanism of both cell survival and cell death, and its role and regulation are of significant interest. The beclin 1 (BECN1) gene, a mediator of autophagy, is deleted in 50% of breast tumors. In primary breast cancer cells resistant to apoptosis, induction of autophagy results in cell death, and heterozygous knockout of the BECN1 gene leads to accelerated malignancy.[49] On the other hand, recent studies point to a role for autophagy in AE resistance, wherein inhibition of autophagy leads to sensitization of resistant cells to AE and subsequent cell death.[50−52] Thus, autophagy may play a dual role, and whether the induction of autophagy is pro- or anticancer is dependent on the cellular context and the stage of cancer development.[51]

Kinases act as connectors of upstream signaling to downstream transcription factors and are central to modulating the effects of growth factors, hormones, and cytokines on the breast epithelium. They are key players in determining the cell's decision to live or die. The ERK/MAPK and PI3K/AKT pathways play an important role in linking the GFR and ER signaling. Kinase signaling is significantly altered during breast cancer development, and its inhibition can reduce cancer cell growth.[53] Ultimately, the kinases converge on key transcription regulators, Jun/Fos for the MAPK and mTOR for the PI3K (Figure 2). The function of transcription regulators in a cancer cell is to overcome self-limiting growth and evade cell death. Evasion of apoptosis by increased expression of anti-apoptotic/pro-proliferative molecules (e.g., BCL2, BCLxl, BCLw, and cyclins A, B, and D) and down-regulation of pro-apoptotic/antiproliferative molecules (BAD, BAX, BIK, and p53) are commonly seen in cancer cells.

EFFECT OF BERRY POLYPHENOLS ON CELL DEATH

If the goal of the cancer cell is to avoid cell-death mechanisms, then the objective of a chemopreventive agent is to enforce these. Data summarized in Table 5 suggest that many berry compounds can induce both apoptosis and autophagy.

Although all of the studies have not been done in breast cancer cells, the aim of this section is to highlight the mechanisms by which berry compounds cause cell death. Because autophagic cell death has not been extensively studied for the selected berry polyphenols in breast cancer cells, evidence from other cell lines is presented. Several mechanisms have been proposed regarding the apoptotic effect of these compounds, whereas ROS generation has been suggested for compounds including delphinidin, kaempferol, and resveratrol; caspase- and p53-dependent apoptosis can be induced by other compounds (Table 5). Regardless of the mechanisms by which these compounds induce cell death, kinase signaling is involved in most cases, except for cyanidin and quercetin (Table 5). Although a likely mechanism is the inhibition of TKRs and the subsequent phosphorylation of downstream kinases (Table 3), it appears that in some cases the opposite is true. For example, delphinidin-induced apoptosis in HepG2 cells involves increased p-JNK,[54] and resveratrol and kaempferol induce apoptosis in breast cancer cells by sustained ERK1/2 activation.[55,56] Berry compounds also induce autophagy by different mechanisms (Table 5). AE-resistant cells may utilize autophagy as a mechanism of cell survival.[51,57] Feng et al. showed that although delphindin has no effect on HCC cells at 24 h, sustained induction of autophagy ultimately results in reduced cell survival at 120 h.[58] Pterostilbene, a blueberry stilbene, induces both apoptosis and autophagy in drug-resistant bladder cancer cells.[59] Because the utilization of autophagy for cell survival is highly dependent on cellular context, it is imperative to explore the effects of berry compounds on autophagy and how the interplay affects the development of drug resistance in breast cancer cells.

LIMITATIONS OF IN VITRO MODELS TO STUDY BREAST CANCER PREVENTION

In vitro models available to study breast cancer usually consist of cancer cell lines that are derived from tumors of varied origins and kept in culture continuously. Although these models are helpful in describing the molecular mechanisms of breast cancer and in the discovery of drugs used to effectively target particular pathways, they fall short when it comes to studying cancer prevention. The reason is that cancer cell lines are already transformed and thus do not represent a typical population for "primary prevention". Immortalized normal cells can be cultured with physiological concentrations of polyphenols for several passages and then tested for transformation upon challenge with a carcinogen. Although this would simulate primary prevention, it must be kept in mind that even these "normal" cells have altered their molecular behavior to adapt to the culture conditions and hence are poor substitutes for mimicking the real nature of the developing breast epithelium. In vitro studies also do not take into account interaction between various cell types in the breast. Various 3-D cultures have been used to mimic the stromal−epithelial interactions.[60,61] However, we are not aware of any study that has used a 3-D culture to study berry polyphenols.

Another limitation of many in vitro studies reported is their use of concentrations of pure compounds in the micromolar range (Tables 3−5). Yet, it is clear that the actual plasma concentrations of many of these agents are in the nanomolar range (Table 1). Thus, the doses used in these studies largely limit their usefulness in the translational setting. The primary paradigm of research with bioactive compounds has been to find agents that "kill" cancer cells, regardless of the concentration at which this occurs. It is easy to understand that killing a cancer cell is not the same as preventing cancer development. Nonetheless, this flawed concept has often supported the use of high in vitro concentrations. Furthermore, due to their relatively nontoxic nature, the maximum tolerated dose (MTD) for many food bioactives is high in rodents.[62] This has in turn supported the use of high doses of a purified compound, rather than a natural food source, in animal models. The culmination of this paradigm is evident in the clinical outcome of the ATBC trial, wherein supraphysiological doses of vitamins A (30 mg) and E (25000 IU) caused an increase in lung cancer incidence in an at-risk population.[63] This has led to a critical rethinking of our approach to food and food component research.[64−66] Thus, currently there is a heightened awareness among scientists to use more physiologically relevant concentrations and an appreciation for the synergy among food components as well as the effects of a food matrix.

Berry extracts form an intermediate system between testing individual berry compounds in vitro and whole berries in vivo. Typically, alcohol extracts enriched in berry polyphenolics have been tested on various cancer cell lines to assess their effects in reducing cell proliferation. The IC_{50} values for these extracts for various cell lines range from 27 μg/mL to 4 mg/mL.[67,68] Studies show that extracts reduce cell proliferation using similar mechanisms of action as pure compounds but at a much lower concentration.[69−71] Extracts also may account for the synergistic action of different compounds as present in the food matrix. Seeram et al. showed that extracts and fruit juices achieve significantly higher antiproliferative effects on various cancer cells than when their individual components are used.[72,73]

EFFECT OF METABOLITES ON THE BIOLOGICAL ACTIVITY OF BERRIES

The complexity of studying berry phytochemicals is further increased by the presence of many metabolites. The metabolism of polyphenols plays an important role in their bioavailability. Also the gut (gastric, intestinal, and colonic) and liver metabolites affect the effectiveness of these compounds in vivo. Gut metabolites of ellagic acid, urolithins A and B, interact with the ER signaling in very different ways (Table 3). The anthocyanidins are conjugated with many types of sugar moieties to form anthocyanins. This can influence both the gut metabolism and the absorption of these compounds.[74] Furthermore, the type and quantity of metabolites generated varies widely depending on the gut microbiota and the gene polymorphisms of the consumer. Only a few of these metabolites have been discovered. Thus, an in vitro study reporting the effects of a pure berry compound will provide only a partial picture of its full potential. The best way to take into account the contribution of metabolites is to use in vivo approaches.

In Vivo Models for Studying Breast Cancer Prevention. Animal models are necessary tools for understanding the effects of both pure compounds and whole berries, given through the diet, on mammary tumorigenesis. Models currently being used consist of 7,12-dimethylbenze(*a*)anthrazene (DMBA)-induced tumors in Sprague−Dawley (SD) rats, E2-induced tumors in August−Copenhagen−Irish hooded (ACI) rats, and transgenic mice containing specific gene alterations and xenograft of human breast cancer cells in immunodeficient mice.[75−78] Although they provide a better system for evaluating the preventive aspects of dietary components, certain key

Table 6. Summary of Three in Vivo Studies Performed with Berry Intervention in ACI Rats

				% reduction compared to control diet[a]									
				2.5% black raspberry diet[b]			2.5% blueberry diet			400 ppm ellagic acid diet			
study	serum E2 level (pg/mL)	implant size (mm) (E2 dose, mg)	length of treatment (weeks)	V	M	B	V	M	B	V	M	B	refs
1[b]	NA[c]	30 (27)	24	76	33	60	70	0	84	37[b]	0	67[b]	92
2	236 ± 24	30 (27)	24	69	40	67	40	0	81	75	43	70	93
3	200 ± 44	12 (9)	32	56	41	41	46	38	43	45	38	47	91, 100

[a]V, tumor volume (mm^3); M, tumor multiplicity; B, burden = tumor volume/multiplicity. [b]Study 1 was a pilot study; some data for ellagic acid group are missing, and hence the true effect of intervention cannot be clearly assessed. Also, the first column is 2.5% mixed-berry diet (0.5% each of blackberry, black raspberry, blueberry, red raspberry, and strawberry) instead of 2.5% black raspberry diet. [c]NA, not available.

points have to be kept in mind regarding the use of these in vivo models in chemoprevention studies. First, in rat models of mammary tumorigenesis, it is important to deliver the initiating insult (DMBA or E2) to the terminal end buds, which are the most susceptible structures, at a specific moment of glandular development. In the DMBA model, this is a very narrow period (ca. postnatal day 50 ± 2), beyond which tumor incidence is greatly altered. In ACI rats, this window is slightly larger (7−8 weeks); however, dramatic differences in tumor volume can still be seen when E2 implantation is done at 7 versus 9 weeks. Dietary phytochemicals fed prior to this window (as typically done in prevention−intervention) can alter mammary gland development and thus potentially change this window. Second, breast cancer xenografts cannot be used for prevention studies because they assess the growth retardation of already tumorigenic cells, and studies in transgenic mice usually focus on a single gene or molecular pathway. Third, the bioavailability of various berry polyphenols changes depending on the species used.[79,80] These factors may influence the data derived from rodent studies, and investigators must be aware of these when they are interpreting chemoprevention data.

Berries in the Prevention of Primary Mammary Tumors in Vivo. Individual berry compounds and whole berries have been used in animal models to illustrate their effect in preventing or reducing tumor growth. Pterostilbene (10 μM) reduces the formation of DMBA-induced mammary transformation in an ex vivo organ culture.[81] Lariciresinol (LR) is a precursor of secoisolarisiresinol, a lignan found in some berries.[82] Saarinen et al. treated rats with 3 or 15 mg/kg of LR 10 weeks after mammary tumors were induced with DMBA. Whereas there was no reduction in incidence or multiplicity of these already established tumors, LR significantly reduced tumor growth and surface area after 9 weeks of administration.[83] Dietary LR (100 mg/kg) also reduces the growth of orthotopic MCF-7 tumors in nude mice.[84,85] Quercetin shows a dose-dependent reduction of 30−50% of DMBA-induced mammary tumors, at dietary doses of 2 and 5%, respectively.[86] However, another study in the same model with a similar treatment protocol showed no effect of a 2% diet.[87] In a recent study, Singh et al. showed that 2.5% quercetin in the diet can increase E2-induced mammary tumors by inhibition of the phase II P450 enzyme catechol-*O*-methyl transferase (COMT) that detoxifies harmful E2 metabolites.[88] These authors clearly show that both phase I and II metabolism of E2 play an important role in the development of E2-induced mammary tumors in ACI rats.[89] However, the dose of 2.5%, translating to 25 g/kg diet, is relatively high. Considering the fact that the highest amount of quercetin to be found in any berry species is only 158 mg/kg in bog whortleberry,[90] such doses are unachievable using natural berry sources. This also underscores the importance of using biologically relevant doses in animal models. Although individual compounds are effective at various levels in reduction of mammary tumor indices, the question of the food matrix effect remains. Whole berries (blueberry and black raspberry) supplemented at 2.5% (w/w) dose inhibit E2-induced mammary tumorigenesis in ACI rats.[91−93] The interest in berries as a chemopreventive agent stemmed from the pioneering work of Stoner and colleagues in using berries for the prevention of esophageal cancer.[94−96] When the ACI rat studies were initiated, whole berries had not been tested in any cancer other than gastrointestinal tract cancers. Nevertheless, sound scientific and translational reasoning lay behind the choice of the type of berries, their respective doses, and the preclinical animal model used.

In initial studies, ellagic acid showed a dose-dependent decrease in 4-hydroxyestradiol-mediated DNA damage in vitro as well as a significant up-regulation of DNA damage repair genes in vivo.[97] Black raspberries had been tested successfully against carcinogen-induced esophageal tumors[98] and are an excellent source of ellagic acid (>1500 ppm) and anthocyanins (≈7000 ppm) primarily in the form of cyanidin glycoside conjugates.[76] On the other hand, blueberry contains moderate levels of anthocyanins (≈4000 ppm) derived from five different anthocyanidins, delphinidin, malvidin, petunidin, peonidin, and cyanidin, but almost no ellagic acid (<100 ppm). They are also cultivated at a much larger scale than black raspberries and are more easily available to the consumers.[99] To further delineate the chemopreventive effects of anthocyanins versus ellagic acid, a group of rats were fed ellagic acid (400 ppm) alone. The equivalent concentration of ellagic acid in 2.5% (w/w) black raspberry diet is 80 ppm; thus, the ellagic acid only diet also compares the effectiveness of delivering a whole food matrix versus individual components. A dose of 2.5% (w/w) for the berries was selected to enable translation to reasonably achievable human doses and is the lowest dose of berries to be tested in an animal model to date. With regard to the animal model, ACI rats implanted subcutaneously with silastic implants containing E2 develop mammary adenocarcinoma in 6−8 months.[93,100,101] The plasma levels of E2 and progesterone (Pg) in these animals are between 35 and 230 pg/mL and 35 pg/mL, compared to 15−53 and 35 pg/mL, respectively, in untreated rats.[93,100,102] In premenopausal women, the E2 levels range between 20 and 1500 pg/mL depending on the ovulatory phase, whereas the progesterone levels are 1−9 ng/mL during ovulation with a very wide interindividual variation.[103] Because higher circulating levels of these steroid hormones are linked to

increased breast cancer risk,[104,105] this preclinical model is to some extent clinically relevant. Furthermore, the combination of low-dose dietary berries and high-dose E2 provides a higher translational quotient.

Dietary black raspberry, blueberry (2.5% w/w), and ellagic acid (400 ppm) significantly reduce E2-induced mammary tumor indices such as tumor latency, incidence, volume, multiplicity, and mortality to various extents in the ACI rat model. The data from three individual tumorigenesis studies summarized in Table 6 show that these berries are consistently effective in the prevention of mammary tumors in this animal model. Black raspberry is the most effective in reducing tumor indices, followed by ellagic acid and then blueberry diet. However, in studies 1 and 2, in which mortality due to pituitary hyperplasia was higher, blueberry significantly reduced this mortality.[93] Berry diets decrease E2-induced mammary cell proliferation in these rats,[106] which may be due to either the promotion of cell death or an antiestrogenic effect. There is evidence for the latter because both dietary berries and ellagic acid counteract the E2-induced increase in the uterine weight.[76] Of interest pertaining to the previous discussion of quercetin, Aiyer and Gupta showed that dietary berries and ellagic acid decrease the expression of COMT in the mammary epithelium at 18 weeks after E2 treatment but not at 6 weeks. This temporal effect is linked to the expression of CYP1A1, an enzyme whose product (2-hydroxy-E2) is the substrate for COMT. CYP1A1 expression is significantly down-regulated by berries at 6 weeks.[91] Even though COMT expression is reduced, there is no increase in tumor indices as reported by the previous authors.[88,91] This further highlights the importance of the food matrix as well as the use of a physiologically relevant dose. Berries also reverse E2-induced hepatic oxidative DNA damage.[107] However, the effect of dietary berries on hepatic enzymes involved in E2 metabolism has not been explored yet.

More recently, Wu et al. have shown that maternal exposure to 5% blueberry diets significantly affects both mammary branching and the size of terminal end buds in pups. This is indicative of a reduced mammary tumor susceptibility[108,109] However, these investigators have not yet reported mammary tumor incidences in these pups. In another study, blueberry juice (100 μL/day; gavage) significantly inhibit the growth of MDA-MB-231 tumors in nude mice mediated by the inhibition of the PI3K/AKT pathway.[70] In a follow-up study, 5 and 10% blueberry diets reduce the volume of orthotopic MDA-MB-231 tumors by 75 and 60%, respectively, suggesting that a lower dose is more effective. Inhibition of Wnt signaling is involved in the tumor retardation.[110]

These data collectively show that whole berries and berry constituents have significant preventive effect on the development of mammary tumors in preclinical animal models. Allometric conversion to human equivalents from the rodent studies suggests that a daily intake of as little as 1−2 cups of fresh berries may provide significant benefits.[76,110] These preclinical studies offer strong evidence to pursue a pilot clinical trial among women at high risk of developing breast cancer. Although long-term follow-ups through the course of a lifetime are not feasible, changes in biomarkers after short- to medium-term exposures can be assessed. The period between initial diagnostic core biopsy and the surgical removal of tumor has been suggested as a potential window to evaluate the effects of chemopreventive agents on breast tissue biomarkers.[111] This window can be effectively used to assess the effect of different types of berries on biomarkers of breast cancer risk.

PERSPECTIVE: BERRIES IN THE PREVENTION OF BREAST CANCER

To put the information gathered from the interactions of berry phenolics with cell-signaling pathways in the context of breast cancer prevention, we will consider the three "windows" of prevention available during a woman's lifetime.[84] The primary prevention window occurs during in utero to peripubertal periods. Epigenetic modification in the mammary gland during this developmental period has an effect on breast cancer risk.[112−115] Furthermore, exposure to dietary phytoestrogens during this developmental period affects breast cancer risk.[116] The only support for effectiveness of berries in this prevention phase comes from a study showing that in utero blueberry diet reduces the number of terminal end buds, the glandular structures most susceptible to transformation by a carcinogen.[109] Epigenetic regulation is a possible mechanism by which this is accomplished. At present, only ellagic acid has been shown to inhibit DNA methyltransferase, an enzyme involved in the methylation of DNA and hence causing epigenetic modifications in MCF7 cells.[117] Data on the effect of other berry polyphenols in the epigenetic regulation of mammary gland development is yet to be explored. Although this may be a good approach to chemoprevention, it may not be directly applicable to the larger at-risk population that currently exists. More studies on the effects of dietary berry exposure at other stages of mammary gland development (in utero, prepubertal, peripubertal) are needed to assess which may be the best stage for preventive intervention.

The secondary prevention window occurs from adulthood to the first diagnosis of breast cancer. Much of the data discussed in this review pertain to this window. Although there is no direct evidence that berry consumption throughout adulthood will prevent breast cancer incidence, the mechanistic action of berry compounds provides support for this. The data from in vitro and in vivo studies suggest that berry polyphenols act in an antiestrogenic fashion in the presence of E2 (Table 3).[76] Berry polyphenols inhibit GFR activation, which is also involved in the growth of primary tumors. These compounds also beneficially modify many other pathways involved in breast cancer development. Preclinical studies presented in the previous section provide evidence to support this. Thus, consuming berries during adulthood could be protective for high-risk women (early menarche, late menopause, high premenopausal circulating E2). Clinical trials can be conducted in high-risk women, and the changes in circulating E2 levels, antioxidant status, plasma and urinary polyphenols/metabolites, and other relevant biomarkers can be assessed as an indication of risk and benefit.

There are many fewer preclinical data available on whether berries will be effective during the tertiary prevention phase, the period after breast cancer diagnosis, when a woman is on adjuvant or neoadjuvant endocrine therapy. This is the chemoprevention of recurrent breast cancer. So far, an animal model that mimics endocrine resistance development is not yet available to researchers. However, on the basis of the signaling effects and what is known about AE resistance development, one can speculate the effects of berry and its compounds in tertiary prevention of breast cancer. If these agents potentiate drug activity, perhaps a lower dose can be used, leading to reduced drug side effects and/or resistance. Finally, the cross-

talk between GFR and ER signaling plays an important role in the development of AE resistance. Inhibition of GFR/kinase signaling by berry polyphenols may reduce the development of resistance arising due to this crosstalk. The effect of polyphenols on the metabolism and clearance of tamoxifen is also an area that requires further research. However, these are only the speculated benefits of the berries. The effectiveness of berries in the tertiary prevention phase still needs to be explored. There is a paucity of data even at the in vitro level, because few studies have focused on how berry polyphenols may affect AE activity. This research is even more imperative because, in the Internet era, much information is available to the public regarding the possible "beneficial" effects of these compounds that may or may not be substantiated by original research.[118] The onus is on the scientific community to provide persuasive evidence for the protective effect of berries and berry polyphenols in the prevention of recurrent breast cancer, especially when provided alongside routine endocrine therapies.

Berry bioactives have a high potential for breast cancer chemoprevention as they act on multiple pathways involved in carcinogenesis. Studies from preclinical animal models strongly support the preventive role of berries in primary breast cancer. Interaction with the ER and TKR signaling may play a role in this beneficial effect. However, their effect on other pathways such as inflammatory and angiogenic signaling cannot be dismissed. Berries may also reduce tumor growth by promoting cell death. Their role in inducing apoptotic cell death is well studied. However, autophagic cell death is emerging as an important mechanism by which a population of cells can survive drug treatment and thus lead to drug resistance. The study of how various berry polyphenols affect autophagy during the development of AE resistance can illuminate their usefulness for the prevention of drug resistance.

Finally, there are very limited data in the literature for the anthocyanins and anthocyanidins. Considering that anthocyanins are the most ubiquitous polyphenols present in berries and likely play an important role in the in vivo effects of whole berries, more research is required on how anthocyanins interact with and alter the various molecular pathways of breast cancer development.

With heightened media awareness regarding a healthy lifestyle, berries are fast becoming the go-to superfruits for their health benefits. On the basis of the evidence presented, the case for using berries as preventive intervention in primary breast cancer is strong; whether this is true in recurrent cancer remains to be determined. There is much that needs to be explored before firm recommendations can be set forth for breast cancer survivors.

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