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Breast cancer and sphingolipid signalling

Paola Signorelli and Riccardo Ghidoni*

Laboratory of Biochemistry and Molecular Biology, San Paolo University Hospital, School of Medicine, University of Milan, 20142 Milan, Italy

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Introduction

Cancer develops from overlapping events that tend to deregulate the metabolism and unbalance the homeostasis of cells. Sphingolipids, major components of biological membranes, are also mediators of intracellular signalling. Their metabolism can be influenced by diverse stimuli and the accumulation or deficiency of intermediates may trigger proliferation and/or impair the ability of damaged cells to undergo apoptosis. Many sphingolipid-regulated functions are implicated in tumour initiation, promotion, progression and responsiveness to chemotherapy. In this review, evidence of the alteration of sphingolipids metabolism and signalling will be discussed in breast cancer prevention and therapy.

Pathophysiology and therapy of breast cancer

Mammary epithelium has a cyclic development during the oestrous cycle, with proliferative phases controlled by oestrogens and progesterone, growth factors and extracellular matrix interactions and a limited and specific epithelial cell regression occurs to maintain tissue size homeostasis (Andres & Strange, 1999). Similarly, mammary gland development during puberty and tissue remodelling after lactation to prepare the gland for successive pregnancy, require a controlled process of apoptosis and matrix degradation. These programmed cell death mechanisms are triggered by different stimuli (e.g., Fas-L, IL6, IGF-1, TGFbeta and the activation of Eph family of tyrosine-kinase receptors) and intracellularly mediated by multiple factors such as Bcl2 family members, p53, caspases and the transcriptional regulators AP1 and NF-kB (Andres & Strange, 1999; Green & Streuli, 2004). Their deregulation may be a leading cause of cancer initiation and promotion.

Breast epithelium expresses steroid receptors and cell proliferation, differentiation and metabolism are regulated by hormones. In postmenopausal women, the fall in ovarian oestrogens may enhance survival strategies and resistance to stresses. Therefore oestrogen replacement can revert cellular metabolism and high dose of oestrogens have been shown to cause tumour regression in preliminary clinical studies (Lonning, 2001; Jordan, 2004). At the same time, extraovarian oestrogens can be produced by adipose tissue and normal or malignant breast tissue under stimulation of cytokines (e.g., TNFalpha) and growth factors, thus promoting iperplasia and neoplasia (Purohit et al. 2002). This applies to approximately 80% of breast cancers, which are oestrogen receptor positive (ER+: mainly ERalpha but also ERbeta). A therapeutic approach is offered by Selective Estrogens Receptor Modulators (SERM) that may provide agonistic and antagonistic effects, depending on the hormonal status of the tissue (Jordan, 2004). The remaining hormone-independent tumours are associated with a higher rate of proliferation and metastatization, less differentiation and non-responsiveness to common therapy (Keen & Davidson, 2003). Consequently anti-tumour strategies must rely on targeting epigenetic and post-transductional mechanisms involved in cancerogenesis and cancer progression. Whole metabolic pathways have been found enhanced or down-regulated in breast cancer, owing to aberrant activity of nuclear receptors and other transcriptional factors (RAR, RXR, VDR, PPAR, AP1, NF-kB, STAT), of proteins such as growth-factor receptors, Ras-Raf-MAPK, PI3K-AKT/PKB or aberrant expression of inhibitors of apoptosis and Bcl2 family members (Keen & Davidson, 2003). Many chemotherapeutics are addressed to such intracellular targets that are known to be regulated by sphingolipid bioactive molecules and sphingolipid-induced pathways (Ogretmen & Hannun, 2004).

Sphingolipids in normal and in cancer cells

Sphingolipid synthesis starts in the endoplasmic reticulum where palmitoyl-CoA and serine are condensed to the sphingoid base, 3-keto-dihydrosphingosine, by serine palmitoyl transferase (SPT), and reduced to dihydrosphingosine. The addition of long chain (14–26 carbons), saturated or unsaturated fatty acids by ceramide synthase yields dihydroceramide, a non-bioactive intermediate that

*For correspondence; e-mail: riccardo.ghidoni@unimi.it

is desaturated to the signalling mediator ceramide. Ceramide can be conjugated with glucose in the Golgi apparatus by glucosylceramide synthase (GCS), and successively with other monosaccharides, such as galactose, N-acetylhexosamines, fucose and sialic acid, by specific glycosyltransferases; or with phosphocholine to form sphingomyelin (SM) by SM synthase, either at the plasma-membrane or in the Golgi. Ceramide is released by the hydrolysis of SM at the plasma membrane (neutral sphingomyelinases, nSMase) or in lysosomes (acidic sphingomyelinase, aSMase), or by the hydrolysis of glucosylceramide in the lysosomes. Ceramide can also be cleaved by ceramidases (acidic CDase in the lysosomes and alkaline and neutral CDases) into fatty acid and sphingosine. The latter is re-acylated by ceramide synthase to form ceramide (recycling pathway) or it can be phosphorylated to sphingosine-1-phosphate (S1P), which is subjected to dephosphorylation by S1P phosphatase or degradation by S1P lyase (Pettus et al. 2002).

Sphingolipid metabolism maintains the equilibrium among different molecules that are inter-converted one to another and that are components of cellular structure as well as regulators of differentiation, senescence, proliferation and cell death (Ogretmen & Hannun, 2004). Upon stresses, pharmacological treatment or physiological stimuli, the apoptotic sphingolipid mediator ceramide can accumulate either by release from sphingomyelin (Strum et al. 1994; Santana et al. 1996; Dbaibo et al. 1998; Zhang et al. 2001; Luberto et al. 2002; Heinrich et al. 2004; Lee et al. 2004) or by an increased rate of its formation *de novo* (Bose et al. 1995; Biswal et al. 2000; Perry et al. 2000; Dbaibo et al. 2001; Chalfant et al. 2002) and decreased rate of its metabolism (Bleicher & Cabot, 2002) (Fig. 1). Ceramide affects, either directly or indirectly, multiple intracellular targets promoting cell growth arrest and/or apoptosis: inhibition of Raf, AKT/PKB, classical PKC, activation of KSR, proapoptotic PKCζ, stress-related kinases JNK, phosphatases (PP1, PP2A) and their target oncogene Rb, phospholipases (PLD), cathepsin D. Ceramide generation leads to translocation and modulation of the activity of Bcl2 family members, altering mitochondrial functions, and to the activation of the apoptosis executioner caspases (Ruvolo, 2003). Being a highly hydrophobic molecule, ceramide formation is most probably confined to membrane compartments and this endows specificity of action to differently formed ceramide pools (Signorelli et al. 2001), with the mitochondrial ceramide being a powerful apoptotic inducer (Birbes et al. 2004).

Sphingolipid mediators show a specificity of action that depends on stressing agents and cellular ability to counteract it. Ceramide can be metabolized to glucosylceramide (Hannun & Obeid, 2002) or even driven towards survival pathways that use ceramide to form molecules that are involved in cellular growth such as SM (Marchesini et al. 2004) and S1P (Cuvillier & Levade, 2003) (Fig. 1). S1P, which is present in cells in very low concentrations, increases rapidly and transiently in response to growth factors and serum and promotes proliferation. S1P can be released and functions as a paracrine factor, engaging G-protein coupled receptors Edg (Spiegel et al. 1994; Spiegel & Milstien, 2000).

Uncontrolled proliferation rate due to oncogenes or pro-survival proteins overexpression (e.g., Rb, Bcl2) or oncosuppressors or apoptotic proteins downregulation (e.g., p53, BAX) can be counteracted by induction of sphingolipid mediators that restore the apoptotic programme. Therefore pharmacological modulation of sphingolipid metabolism may offer a new chemotherapeutic strategy against tumours, including breast cancer (Cuvillier & Levade, 2003; Kester & Kolesnick, 2003; Ogretmen & Hannun, 2004).

It has been shown recently that supplementation of animal diets with dairy glycosphingolipids and ceramides dramatically reduces genetic and pharmacologically induced digestive tract cancer (colon) (Schmelz et al. 2000). The development of new techniques for sphingolipid delivery at the tumour site, such as liposome vesicles, may offer new perspectives for therapy of breast and other types of cancer (Shabbits & Mayer, 2003; Stover & Kester, 2003) Conversely, alteration of sphingolipid metabolism can also elicit survival signals thus favouring tumourigenesis and acquisition of drug resistance (Senchenkov et al. 2001). Reduction of endogenous ceramide was measured in human colon cancer relative to healthy colon mucosa (Selzner et al. 2001). Drug resistance to chemotherapeutics is related to increased expression and activity, in cancerous *v.* normal cells, of enzymes involved in sphingolipid metabolism and the overexpression or silencing of such enzymes was shown to modulate drug sensitivity (Senchenkov et al. 2001; Cuvillier & Levade, 2003; Kester & Kolesnick, 2003; Ogretmen & Hannun, 2004).

Enzymes of sphingolipid metabolism in breast cancer

Ceramide is the structural core of complex sphingolipids such as sphingomyelins and glyco-ceramides. Recently a neutral sphingomyelinase was shown to be associated with cellular proliferation mechanisms in breast cancer cells (Marchesini et al. 2004). This enzyme was differently activated throughout cell cycle phases and both protein expression and activity were up-regulated by cell-to-cell contact. Cell-to-cell contact inhibition is one of the control mechanisms that is altered in tumour growth and progression. In this sense nSMase may be considered a tumour growth suppressor (Marchesini et al. 2004). nSMase activation in response to TNFalpha treatment yields an increase in ceramide and apoptosis (Luberto et al. 2002; Birbes et al. 2005).

Breast cancer cells with acquired drug resistance and breast tumours from patients resistant to chemotherapy exhibited a glucosylceramide intracellular content higher than that detected in sensitive cells or tissues from

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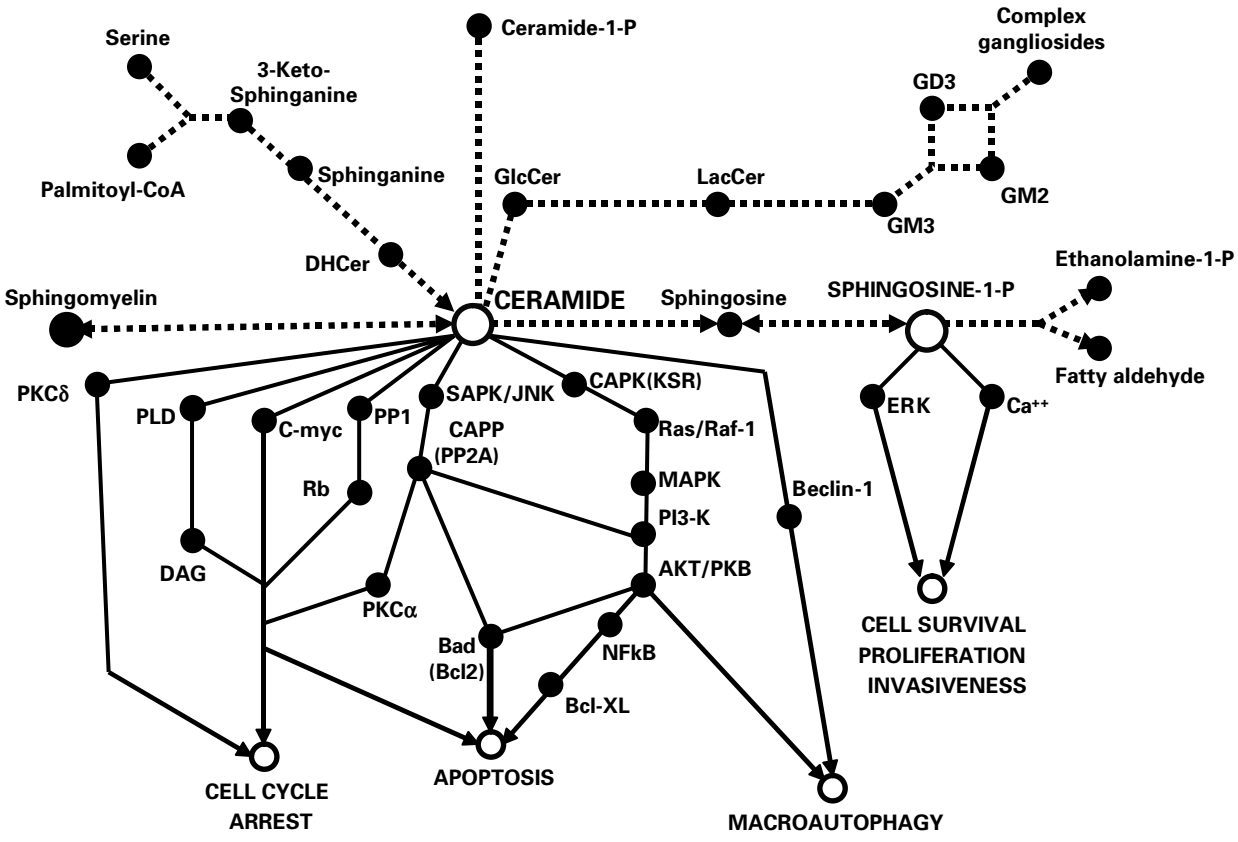

Fig. 1. A ''subway'' map of sphingolipid metabolism and signalling.

therapy-responsive patients (Lucci et al. 1998). In breast cancer, increased activity of GCS shifts ceramide accumulation to formation of glucosylceramide (Lavie et al. 1996; Lucci et al. 1998; Liu et al. 2001; Gouaze et al. 2004; Liu et al. 2004) thus enhancing tumour resistance to apoptotic stresses that are ceramide-mediated. An enhanced expression and activity of GCS has been associated with increased expression of MDR-genes products and resistance acquisition in breast cancer cells (Gouaze et al. 2004).

Human glycanase (endoglycosyl ceramidase) cleaves glycosphingolipids into ceramide and oligosaccharides (Basu et al. 1998). Its activity is modulated during gestation and lactation in rats (Basu et al. 1997). Glycanase activity detected in breast and other cancer cells is lower than that present in normal tissues (Basu et al. 2000). These observations suggest that glycanase down-regulation may play a role in tumour promotion, by lowering intracellular ceramide levels derived from glycosphingolipid turnover.

Exogenously administered sphingosine-1-phosphate (S1P) enhanced breast cancer cellular growth (Goetzl et al. 1999; Wang et al. 1999a; Nava et al. 2002), reduced motility (Spiegel et al. 1994; Wang et al. 1999a) and promoted drug resistance (Nava et al. 2002; French et al. 2003). In agreement with these observations, the expression of S1P-receptors Edg2 and Edg3 in breast tumour cells increased significantly the proliferation rate in response to S1P, by a G-protein dependent mechanism (pertussis toxin inhibitable) (Goetzl et al. 1999). Additionally S1P seems to act either via receptor triggering or via unknown intracellular targets, since endogenously produced S1P in breast cancer cells was shown to mediate signalling independently from Edg expression (Wang et al. 1999b).

Breast tumour biopsies from patients were reported to have a significantly higher expression of sphingosine kinase (SPHK) than the adjacent normal mammary epithelium. Similarly, syngenic mammary tumour cells, inoculated into mice, revealed an increased SPHK activity than that associated to non-transformed cells of the host (French et al. 2003). Thus SPHK may be considered as a tumour-promoting enzyme, conferring on cells a proliferative phenotype. Data confirming this hypothesis show that overexpression of SPHK in breast cancer cells markedly increased endogenous levels of S1P. The endogenous S1P was not extracellularly released, and it activated pathways leading to increased proliferation rate, in full serum cultured cells (Wang et al. 1999b). A proposed target for endogenously accumulating S1P, in breast cancer cells overexpressing SPHK, are the Extracellular Activated Kinases (EAK), whose activation was required for the increase in proliferation (Wang et al. 1999b). Intriguingly,

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SPHK overexpression potentiated oestrogen mitogenic stimulus and tumour promotion without affecting oestrogen receptor expression nor regulation of oestrogen-responsive genes, suggesting that S1P and hormones trigger independent and synergic effects (Nava et al. 2002). Xenografts of human breast cancer cells overexpressing SPHK in mice mammary fat pads developed significantly faster in larger tumour masses than in wild type breast cancer cell xenografts (Nava et al. 2002).

Sphingolipids and breast cancer metastasis

Sphingolipids (sphingomyelin, gangliosides) and cholesterol are tightly packed in membrane microdomains. These have been proposed to form sites where membrane proteins cluster, thus initiating signalling cascades (Holthuis et al. 2001; Tepper et al. 2002; Gulbins & Kolesnick, 2003). Reorganization of membrane lipids by sphingomyelin enrichment and microdomain formation can modulate signalling events, such as Ras recruitment, growth factors and chemokine receptor polarization, that lead to breast cancer migration (Manes et al. 1999; Keely, 2001; Jaumot et al. 2002).

Hydroxylated cholesterol, a molecule released by osteoblasts, has been shown to trigger an increase in breast cancer cellular sphingomyelin content (Schroepfer, 2000) and this was associated with the acquisition of metastatizing phenotype (Silva et al. 2003).

Exogenous S1P administration to breast cancer cells, at much higher doses than that required for S1P-receptors (Edg) saturation thus acting independently from membrane receptors, activated Focal Adhesion Kinases (by autophosphorylation) and inhibited matrix metalloproteinases (MMP2), modulating cellular invasiveness (Wang et al. 1999a).

Sphingolipids as mediators of chemotherapeutics

Serum levels of TNFalpha increase with breast cancer staging and can be used as a tumour marker (Sheen-Chen et al. 1997). Breast tumours may express death-associated receptor for TNF-ligand family and undergo apoptosis when treated with TNFalpha, TRAIL (TNF-Related-Apoptosis-Inducer-Ligand) (Jaattela et al. 1995; Keane et al. 1999; Herrnring et al. 2000) and to a lesser extent with Fas-ligand (Keane et al. 1996; Turley et al. 1997).

Ceramide was reported to mediate apoptosis in response to TNFalpha in breast cancer cells. The formation of ceramide was shown to depend on the signalling activated by a receptor-associated molecule FADD (Chinnaiyan et al. 1996), to be related to nSMase activation (Liu et al., 1998) or to activation or both neutral sphingomyelinase (nSMase) and acidic sphingomyelinase (aSMase) (Cai et al. 1997). A lower content of sphigomyelin was measured in TNFalpha-resistant mammary adenocarcinoma cells compared with sensitive, in association with the inability to activate sphingomyelin hydrolysis pathways (Cai et al. 1997). This suggests that different compositions of plasma membrane sphingolipids may affect receptor signalling mechanisms (such as SMase activation) thus modulating cancer responsiveness to chemotherapy. In addition, the apoptotic pathway triggered by TNFalpha in breast cancer cells was shown to require not only nSMase activation but also an increase in ceramide derived from synthesis *de novo* (Dbaibo et al. 2001). Pharmacological inhibition of nSMase (and not aSMase) reduced the initial ceramide accumulation and not the late peak due to the synthesis *de novo* but this was sufficient to protect from TNFalpha-induced mitochondrial dysfunction and cytotoxicity, suggesting that specific pools of ceramides are able to activate the apoptotic machinery (Luberto et al. 2002). Anyway, cell variants of a line of breast cancer exhibited different sensitivity to TNFalpha, with an increasing toxicity that was proportional to TNFalpha receptor (p55) expression, Bcl2-family members expression and to the ability to raise ceramide content (Burow et al. 1998). Moreover a high level of expression of anti-apoptotic Bcl2 family members inhibited TNFalpha-induced cell death downstream of ceramide production (Liu et al. 1999b).

Alkylating agents and antimetabolites were the first drugs introduced in breast cancer chemotherapy, followed by anthracyclines introduced about 30 years ago and used often in combined therapy. A decade ago a new class of chemotherapeutics, the taxanes, was successfully applied in mammary tumour clinical trials (Friedrichs et al. 2002). Other successful pharmacological tools recently introduced in breast cancer chemotherapy are inhibitors of oestrogen synthesis (such as aromatase inhibitors) (Miller, 1999) and a class of oestrogen-like molecules, with mixed action as agonist or antagonist (Selective Estrogen Receptor Modulators, SERM) according to tissue expression of oestrogen receptors (Mandlekar & Kong, 2001). Another class of pharmacological agents are all-*trans*-retinoic acid (ATRA), the natural metabolite of vitamin A that reversibly inhibits growth of breast cancer tumours in dependence of retinoid receptor (RAR) status of the tumour (Dawson et al. 1995; Sirchia et al. 2000). N-(4-hydroxyphenyl)retinamide (4-HPR) is a synthetic analogue of ATRA, used in breast tumour therapy whose action is not receptor mediated (Maurer et al. 2000). Etoposide and anthracyclines exibited breast tumoural cell toxicity via ceramide mediated pathways (Gewirtz, 2000; Wang et al. 2002). Doxorubicine induced ceramide accumulation and apoptosis in breast cancer cells via activation of nSMase (Gouaze et al. 2001). Ceramide induced by doxorubicine was reported to be metabolized into sphingosine that acted as an apoptotic inducer. To confirm this conclusion, exogenously administered sphingosine was shown to be toxic to breast cancer cells (Cuvillier et al. 2001).

Overexpression of the antiapoptotic BclxL was able to block completely sphingosine but only partially

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Table 1. Drugs affecting cell death via sphingolipid mediators in human breast cancer

Agent	Cell line	Cell response	SL pool increase	Mechanism of SL increase	Reference
Suramin	MCF-7	Cell death	Exogenous BODIPY-cer	—	(Gill & Windebank, 1997)
TNFalpha	MCF-7	Cell death	Ceramide	—	(Burow et al. 1998)
	MCF-7	Cell death	Ceramide	↑ nSMase	(Liu et al. 1998)
	MCF-7	Cell death	Ceramide	↑ nSMase, ↑ aSMase	(Cai et al. 1997)
	MCF-7	Cell death	Ceramide	↑ nSMase, ↑ *de novo* synthesis	(Dbaibo et al. 2001)
	MCF-7	Cell death	Ceramide	↑ nSMase	(Luberto et al. 2002)
	MCF-7	Cell death	Ceramide	↓ GlcCer synthase	(Liu et al. 1999b)
4-HPR	MDA-MB-468	Cell death	Ceramide	↑ *de novo* synthesis	(Maurer et al. 2000) (Wang et al. 2002)
	MCF-7	Cell death	Ceramide	↑ *de novo* synthesis	(Rehman et al. 2004)
Taxol	MDA-MB-468 /MCF-7	Cell death	Ceramide	↑ *de novo* synthesis	(Charles et al. 2001)
	MDA-MB-468	Cell death	Ceramide	↑ *de novo* synthesis	(Maurer et al. 2000) (Wang et al. 2002)
Doxorubicin	MCF-7	Cell death	Sphingosine	—	(Cuvillier et al. 2001)
	T47D	Cell death	Ceramide	↑ nSMase	(Gouaze et al. 2001)
Etoposide	MDA-MB-468	Cell death	Ceramide	↑ *de novo* synthesis	(Maurer et al. 2000) (Wang et al. 2002)
Safingol	MCF-7	Cell death	—	—	(Sachs et al. 1995)
Daunorubicin	MDA-MB-468	Cell death	Ceramide	↑ *de novo* synthesis	(Wang et al. 2002) (Maurer et al. 2000)
Resveratrol	MDA-MB-231	Cell death	Ceramide	↑ *de novo* synthesis	(Scarlatti et al. 2003)
Tamoxifen	MCF-7	Autophagy	Ceramide	↑ *de novo* synthesis	(Scarlatti et al. 2004)
				↓ GlcCer synthase	(Lavie et al. 1997)
	MCF-7	Cell death	Ceramide	↓ GlcCer synthase	(Lucci et al. 1999)

doxorubicin-induced apoptosis, suggesting that exogenous sphingosine is formed upstream of mitochondria damages (Cuvillier et al. 2001).

Taxanes induce mitotic arrest and apoptosis by interfering with microtubule association thus arresting the cell cycle. Their action in breast tumours was also shown to promote intracellular accumulation of ceramide (Wang et al. 2002) via stimulation of its synthesis *de novo* (Charles et al. 2001). Pharmacological inhibition of ceramide synthesis reduced cell sensitivity to taxol. The IC_{50} of taxol was cell specific and inversely proportional to the ability to raise ceramide intracellular levels (Charles et al. 2001).

SERM act on hormone-responsive and non-responsive breast tumours, either by regulating gene transcription or by affecting intracellular apoptotic mediators. Tamoxifen exhibits differential effects according to ER expression, being a partial agonist for ERalpha and a pure antagonist for ERbeta, but also triggering non receptor-mediated intracellular pathways leading to apoptosis (Mandlekar & Kong, 2001) via ceramide accumulation (Kester & Kolesnick 2003). Tamoxifen suppressed or reduced the shunt to glucosylceramide and gangliosides, thus leading to ceramide accumulation in hormone-responsive (ER+) breast cancer cells (Lavie et al. 1997). Hormone independent (ER−) cell lines were sensitive to a combined treatment of tamoxifen and 4-HPR, exhibiting significant changes in plasma membrane ganglioside composition (Aoyama, 2002). Ceramide synthesis *de novo* was shown to be triggered by tamoxifen and to induce autophagic cell death, a mechanism stimulated by nutrient depletion that raises an initial survival response followed by cell death (Scarlatti et al. 2004).

Recently the phyto-oestrogen, resveratrol, was reported to induce breast cancer cell toxicity by eliciting synthesis *de novo* and accumulation of ceramide. The apoptotic pathway and activation of caspases induced by resveratrol was reversed by pharmacological inhibition of the sphingolipid neo-synthesis (Scarlatti et al. 2003).

4-HPR induced cell toxicity and accumulation of ceramide in breast cancer cells by enhancing its synthesis *de novo* (Maurer et al. 2000; Wang, et al. 2002; Rehman et al. 2004). Pharmacological inhibition of ceramide synthesis was prevented by drug toxicity (Maurer et al. 2000). A list of anticancer drugs acting through sphingolipid signalling is shown in Table 1.

Sphingolipid metabolism in breast cancer resistance to chemotherapy

Acquisition of resistance to multiple drugs (multidrug resistance, MDR) frequently develops in breast cancer during therapy by increased expression of drug transporters,

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enhanced detoxification activities, alteration in topoisomerase activity and in cellular apoptotic pathways (Persidis, 1999; Pommier et al. 2004). Resistance to multiple chemotherapeutics acting via ceramide has also been associated with an enhanced ability to glycosylate ceramide, thus reducing its intracellular content and signalling to the apoptotic pathway (Lavie et al. 1996). Higher levels of glucosylceramide were measured in drug-resistant *v.* drug-sensitive breast tumour cells and in human breast tumour specimens from patients who failed chemotherapy *v.* specimens from patients who responded to chemotherapy (Lucci et al. 1998). An enhanced expression of glucosylceramide synthase (GCS) (Gouaze et al. 2004) was detected in a selected drug-resistant pool of breast tumour cells when compared with GCS level in the original sensitive cell line. Pharmacological inhibition of GCS sensitized MDR/breast cancer cells to diverse chemotherapeutics such as taxol, vincrastine and adriamycin (Lavie et al. 1997; Shabbits & Mayer, 2002), enhanced toxicity of 4-HPR (Rehman et al. 2004) and of tamoxifen (Scarlatti et al. 2004). Similarly the silencing of GCS expression restored drug sensitivity (Liu et al. 2004). Breast cancer cells overexpressing GCS acquired resistance to drugs (adriamycin), to the apoptotic effect of exogenously added ceramides (Liu et al. 1999a) and to ceramide-mediated apoptosis induced by TNFalpha (Liu et al. 1999b).

Under drug selective pressure, the increased expression of GCS in breast cancer cells was paralleled by an increased expression of P-glycoprotein (P-gp) (Gouaze et al. 2004). A well studied mechanism in breast cancer MDR is related to the overexpression of the membrane transporter P-gp, encoded by MDR1 gene, that is able to efflux drugs out of the cell (Glazer & Rohlff, 1994). The anti-oestrogen tamoxifen is also known as P-gp modulator (antagonist) (Callaghan & Higgins, 1995). P-gp has also been proposed as the transporter of glucosylceramide from the cytosolic side of the Golgi to the lumen, for higher glycosylation (van Meer et al. 1999). Tamoxifen reduced significantly glucosylceramide as well as ganglioside intracellular levels in breast cancer cells (Lavie et al. 1997) and impaired the metabolism of short-chain exogenous ceramides (Lucci et al. 1998), reverting cell resistance phenotype.

Ceramide clearance is also due to ceramidase (Cdase) hydrolysis into sphingosine, another highly toxic compound. This can be phosphorylated to form the proliferative mediator S1P. In breast cancer cells overexpression of SPHK led to a marked increase of S1P cellular content (that is normally low) and to the acquisition of resistance to doxorubicin, TNFalpha/Actinomycin D and exogenous sphingosine (Nava et al. 2002). Novel compounds inhibiting SPHK activity showed cellular toxicity even in breast adenocarcinoma cell lines overexpressing drug transporters such as P-gp, suggesting that such inhibitors may be effective also in chemoresistant breast cancers (French et al. 2003). In addition, sphingoid base analogue treatments were shown to be effective in overcoming drug-resistance (Sachs et al. 1995).

Sphingolipid analogues as potential anticancer agents

Given the pivotal role of sphingolipids in the control of cellular functions, a number of synthetic analogues have been tested on tumour cell lines (Cuvillier & Levade, 2003; Ogretmen & Hannun, 2004). Exogenous administration of ceramide analogues has been commonly used to mimic the effects of stresses, inflammatory agents and chemotherapeutics (Hannun & Obeid, 2002). The ability of short-chain ceramide analogues (C2 and C6) to inhibit proliferation and induce cytotoxicity has been assessed in breast cancer cell lines (Cai et al. 1997; Ameyar et al. 1998; Pirianov et al. 1999; Charles et al. 2001; Gouaze et al. 2001; Kundu et al. 2002; Scarlatti, et al. 2004). Short-chain ceramides are synthetic analogues, not naturally occurring, extensively used to overcome the poor solubility and the low efficiency of delivery of the hydrophobic natural long-chain ceramides. The use of unnatural short-chain analogues is debated (Luberto & Hannun, 2000), since they can disrupt sphingolipid clustering in rafts at the plasma membrane, thus affecting cellular signalling (Gidwani et al. 2003). Moreover, there is contrasting evidence on their intracellular metabolism (Rosenwald & Pagano, 1993; Lucci et al. 1999) and in breast cancer they were shown to be converted in glucosylceramides only in MDR-resistant (adriamycin-resistant) and not in drug-sensitive cells (Lucci et al. 1999). A phase II clinical trial for cutaneous breast cancer therapy with topical application of short-chain ceramides (C2 and C6) has been attempted with poor success (Jatoi et al. 2003). The authors question the validity of this therapy, although they applied a very low dose (1%) of ceramides. Moreover the incorporation into cationic or pegylated liposome vescicles can help to achieve a higher efficiency of delivery to cells (Stover & Kester, 2003). Recently the introduction of a second unsaturation in the sphingoid base of ceramide (between C5 and C6) resulted in an analogue with a higher toxicity than ceramide in breast cancer cells but significantly less toxic on non transformed breast epithelial cells (Struckhoff et al. 2004).

Sphingosine, the product of ceramide breakdown, is known to be a potent PKC inhibitor, cytostatic and cytotoxic mediator (Hannun & Bell, 1989; Becker et al. 2004). Treatment with sphingoid bases induced apoptosis in a variety of tumour cell lines (Cuvillier, 2002) and enhanced pharmacological toxicity or overcame radio- and chemo-resistance (Cuvillier & Levade, 2003). D-Erythro-sphingosine induced significantly higher toxicity than L-threo-sphingosine on breast cancer cells, whereas dihydrosphingosines were inactive (Sakakura et al. 1998). Exogenous sphingosine was shown to induce cell death in breast cancer cell lines and pharmacological inhibition of ceramide synthase (that re-acetylates sphingosine to

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ceramide) partially protected cells, suggesting that ceramide can mediate sphingosine-related apoptosis cascade (Cuvillier et al. 2001).

A synthetic analogue of sphingosine, safingol, was reported to partially revert chemoresistance of breast cancer cells by inhibiting PKC-induced phosphorylation of P-gp (Sachs et al. 1995), although phosphorylation of this drug transporter has still unclear effects (Castro et al. 1999). Moreover safingol at subtoxic concentration prompted breast cancer cells towards 4-HPR toxicity. The authors reported a significant increase in ceramide intracellular levels, not further metabolized into glucosylceramide, in cells co-treated *v.* single treated with 4-HPR, suggesting that safingol can be acylated to a non-metabolized ceramide that sensitizes to chemotherapy (Maurer et al. 2000).

Conclusions

In the present review we have outlined the role of sphingolipid signalling in breast cancer. The extensive number of publications offers a starting point for future research of antitumour strategies. These include the design of novel sphingolipid-based drugs that specifically target sphingolipid metabolism by inducing ceramide accumulation, inhibiting ceramide clearance and S1P generation. The coming clinical trials with new-generation anticancer drugs, although still at a very early phase, represent a promising window to open on breast cancer therapy and prevention.

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