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TOPICAL REVIEW

Breast Cancer Proteome Takes More Than Two to Tango on TRAIL: Beat Them at Their Own Game

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Abstract Breast carcinogenesis is a multidimensional disease that has resisted drug-related solutions to date because of heterogeneity, disorganized spatiotemporal behavior of signal transduction cascades, cell cycle checkpoints, cell transition, plasticity, and impaired pro-apoptotic response. These synchronized oncogenic events, including protein–protein interaction, transcriptional–regulatory, and signaling networks, trigger genomic and transcriptional disturbances in TRAIL-mediated signaling network neighborhoods. Therefore, tumor cells often acquire the ability to escape death by suppressing cell death pathways that normally function to eliminate damaged and harmful cells. This review describes the TRAIL-mediated cell death signaling pathways, the interactions between these pathways, and the ways in which these pathways are deregulated in breast cancer.

Keywords Biochemistry/molecular biology · Cell physiology · Cell signaling · Membrane transport · Membrane-drug physical interaction · Structure function membrane rafts · Transport physiology

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Introduction

We now know that multiple signaling pathways from several cell types are orchestrated with mechanical cues and cell rearrangements to establish the pattern of the mammary gland. The integrated mechanical and molecular pathways that control mammary morphogenesis are tightly controlled as a disruption of spatiotemporal behavior that underpins breast carcinogenesis. The progression of cells from a normal differentiated state, in which rates of proliferation and apoptosis are balanced, to a tumorigenic and metastatic state involves the accumulation of mutations in multiple regulators of signal transduction cascades, as well as the evolution and clonal selection of more aggressive cell phenotypes.

Extensive information has been added to the ever-increasing and expanding facet of tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-mediated apoptosis. It initiates binding of Apo2L/TRAIL to their respective death receptors (DRs), which consequently induce their trimerization and form a death-inducing signaling complex (DISC). The resultant downstream events include recruitment of adaptor protein FADD to the DISC through its death domain (DD), which interacts with the DD of the receptors. Furthermore, attachment of procaspase-8 (and -10) to the DISC through homotypic interaction with FADD via particular death effector domains leads to its activation. There are some proteins that negatively regulate TRAIL-mediated apoptosis, like c-FLIP (FLICE-like inhibitory protein), that can compete with caspase-8 for binding to FADD and thus can inhibit caspase-8 activation. It has been noted that recurrent bCSC activity is attenuated after repeated TRAIL and c-FLIP inhibitory treatment (Piggott et al. 2011). DISC-activated caspase-8 initiates a caspase cascade by processing caspase-3 and

succeeding activation of effector caspases. Caspase-8 also cleaves Bid into truncated Bid (tBid), which initiates the mitochondrial pathway, making the membrane permeable to release of cytochrome c and Smac/DIABLO from the mitochondria. Cytochrome c, in collaboration with Apaf-1, forms apoptosome, which results in activation of caspase-9. Smac/DIABLO overcomes the inhibitory activity of X-linked inhibitor of apoptosis protein (XIAP), thus facilitating full activation of caspases-3 and -9, eventually leading to cell death.

TRAIL interacts with its native DRs. These belong to a tumor necrosis factor receptor superfamily that possesses a cytoplasmic DD. DRs regulate imperative operational and homeostatic phases of the immune system. These communicate signals through apical protein complexes, which are nucleated by the DD adaptors FADD and TRADD, to trigger apoptosis. Sequentially, FADD and TRADD also nucleate numerous distal signalosomes, which mediate cross-talk between different DR signaling pathways in cells. Thus, DR signal transducers may provide important nodes of coordination in breast cancer signaling networks. Evidence indicates that mRNA expression of TRAIL receptors is not indicative of their functional protein expression as a result of posttranslational regulation. Moreover, DR4 is shown to be *N*-glycosylated in prostate cancer cells (Yoshida et al. 2007). It is reasonable that both receptors undergo *O*-glycosylation in various tumor cell lines, and that this activity is more pronounced in TRAIL-sensitive cells (Wagner et al. 2007). However, it is intriguing to note that TRAIL DRs, with a higher incidence in DR4, are functionally deficient in some breast cancer cell lines as a result of a decline in cell surface expression. Investigational studies revealed that DR4 deficiency, which may result from defects in its protein-sorting machinery, is consistent with the cellular resistance to anti-DR4 antibody and a decreased sensitivity to rhTRAIL. It is well known that, like other transmembrane proteins, TRAIL receptors appear to be subject to membrane transport and endocytic sorting signals (Austin et al. 2006; Kohlhaas et al. 2007; Ren et al. 2004; Jin et al. 2004). The absence of DR4 or DR5 cell surface expression points toward probable defects within the translational modification processes. The trajectory of DRs was explored by transfecting cells with a plasmid expressing green fluorescent protein (GFP)-tagged wild-type DR4 (GFP-DR4-wt), and its cellular localization was detected by confocal microscopy. Results indicated that MDA-MB-231 cells had a cell surface expression of DR4-GFP-wt; however, it was found exclusively in the cytoplasm of BT474 cells.

This differential and contextual cell surface expression of DRs in cancer cells underscores the fact that protein-sorting machinery plays an important role. In addition, sequence analysis of DR4 protein demonstrates two potential sorting signals that reside within the cytoplasmic domain: EAQC^{337}LL and ^{409}YAML (Zhang and Zhang 2008). These signals are also present in many other receptors, and they have been implicated in regulating the rapid internalization of proteins and targeting the endosomal–lysosomal compartments (Bonifacino and Traub 2003). To further strengthen the concept that these signals are responsible for DR4 sorting, two DR4 mutants, LL$^{337-338}$AA and Y^{409}A, were generated and overexpressed in BT474 cells. Interestingly, the DR4(Y^{409}A) mutant, like the wild-type DR4 protein, was found in the cytoplasm, whereas the DR4(LL$^{337-338}$AA) mutant showed expression almost exclusively on the cell surface. These results emphasize the notion that the dileucine-based sorting signal (EAQC^{337}LL) acted as a key structural determinant in DR4 for its sorting through the clathrin-mediated endocytosis machinery (Zhang and Zhang 2008).

TRAIL-Mediated Signaling in Breast Cancer

Refined research has verified the fact that by grouping breast cancers according to the expression of the established markers estrogen receptor (ER), progesterone receptor (PR), and HER2 (also known as ERBB2), discrete molecular alterations and outcomes can be identified. Similar markers are used as surrogates for the intrinsic subtypes: luminal A subtype is defined as ER+ and/or PR+, HER2−; luminal B subtype is defined as ER+ and/or PR+, HER2+; HER2-related subtype is defined as ER−, PR−, HER2+; and basal-like tumors are defined as ER−, PR−, HER2− (Wirapati et al. 2008; Nielsen et al. 2004). In addition, the cancer stem cell (CSC) concept derives from the information that cancers are aberrant tissue clones whose continuous propagation is vested in a biologically distinct subset of cells that are typically rare. Breast CSCs have gained prominence as a result of advances in defining normal tissue hierarchies, a greater appreciation of the multistep nature of oncogenesis, and improved methods to propagate primary human cancers in immunodeficient mice.

Triple-negative breast cancer cell lines are sensitive to TRAIL, whereas others are relatively resistant. However, WEE1, a cell-cycle checkpoint regulator, suppresses toxicity of TRAIL. Therefore, pretreatment with WEE1 inhibitor or knockdown of WEE1 increased the toxicity of TRAIL in the basal/triple-negative breast cancer cell lines (Garimella et al. 2012). A similar concept was further explored in TRAIL-sensitive triple-negative cell lines that had a mesenchymal phenotype, while the three TRAIL-resistant triple-negative cell lines had an epithelial phenotype. Cell lines with HER2 amplification were sensitive to TRAIL, and none of the five ER-positive cell lines were TRAIL sensitive. Surprisingly, TRAIL-R2 mediated the effects of TRAIL even when both

TRAIL-R1 and TRAIL-R2 were expressed. Further, inhibition of epidermal growth factor receptor (EGFR) enhanced TRAIL-induced apoptosis in TRAIL-sensitive cell lines but did not convert resistant cells into TRAIL-sensitive cells (Rahman et al. 2009). CSCs derived from CRL-2335 and MDA-MB-468 triple-negative breast cancer cells in vitro demonstrated misrepresented Wnt-1 signaling and its downstream target, β-catenin, cyclin D1, increased proliferation, and mammosphere formation. Laboratory investigations indicated that CSCs treated with cisplatin and TRAIL results in a reduction in the mammosphere-forming activity (Yin et al. 2011).

Additional studies have indicated that breast cancer cells have an inactive ERK, c-Jun N-terminal kinase (JNK), p38 MAPK, and hyperactive Cbl-b. More importantly ERK, JNK, and p38 MAPK are involved in up-regulating the expression of DRs. Therefore, the proper activation of these proteins is necessary to trigger the expression of TRAIL receptors. MCF-7 and MDA-MB-231 cells were investigated for proof of concept, and results verified the fact that bufalin significantly sensitized TRAIL-resistant cells to TRAIL-mediated apoptosis (Yan et al. 2012). Similar effects were obtained after treatment of TRAIL resistant breast cancer cells with AT-101, an (−)-enantiomer of gossypol (Kisim et al. 2012). Another antiapoptotic protein documented to be involved in mechanism underlying TRAIL resistance in breast cancer cells is Mcl-1. Treatment of TRAIL-resistant MDA-MB-231 and T47D breast cancer cells with daunorubicin resulted in suppression of Mcl-1 and sensitized resistant cells to TRAIL (Oh et al. 2012). Overexpression of superoxide dismutase (SOD) and XIAP occurs in breast cancer cells. Therefore, this was checked in SUM149, a basal-type cell line isolated from primary tumors. Pretreatment with embelin resulted in activation of ERK-1/2 and reactive oxygen species accumulation, which correlated with the down-regulation of the antioxidant protein SOD1 in XIAP-overexpressing cells. Cells reconstituted for SOD escaped embelin and TRAIL-mediated apoptosis (Allensworth et al. 2012). Survivin is also an apoptotic protein, as inhibition of survivin by siRNA enhances TRAIL-induced apoptosis. In vivo experiments verified that treatment of mice with cisplatin plus TRAIL resulted in a considerable inhibition of CRL-2335 xenograft tumors compared with untreated control tumors (Xu et al. 2011). Wogonin and the structurally similar natural flavones apigenin and chrysin overcome TRAIL resistance by transcriptional suppression of c-FLIP. This is primarily triggered by transcriptional inhibition of the p53 antagonist murine double minute 2 (Mdm2), leading to an increase in p53 levels and then to up-regulation of the p53 target gene *TRAIL-R2* (Ding et al. 2012).

It is interesting to note that elevated MEKK3 expression is found at high frequencies in breast cancer compared to normal breast tissue. Additionally, targeted inhibition of MEKK3 siRNA increased the sensitivity of MCF-7 cells to TRAIL by repressing the transcription activity of nuclear factor-κB (NF-κB) and stimulating the caspase processing to generate executive apoptotic signals (Guo et al. 2012).

Accumulating findings suggest that VCAM-1 tethers metastasis-associated macrophages to cancer cells via counterreceptor α4 integrins. Clustering of cell surface VCAM-1, acting through ezrin, regulates Akt activation and rescues cancer cells from proapoptotic proteins like TRAIL. Contrary to this, the prosurvival function of VCAM-1 can be blocked by antibodies against α4 integrins (Chen et al. 2011). On a similar note, RU486 (mifepristone) greatly enhances TRAIL-mediated apoptosis through suppression of Bcl-2 and c-FLIP(L) as well as CHOP-mediated DR5 up-regulation (Min et al. 2012). Targeted inhibition of MTDH sensitizes cancer cells to cell death induction by TRAIL and histone deacetylase (HDAC) inhibitor LBH589 cotreatment. Abrogation of MTDH resulted in inhibition of PDK1 and Akt phosphorylation along with increased Bim expression and XIAP degradation (Meng et al. 2011).

NF-κB is a transcriptional mediator expressed in the cytoplasm, where its activity is modulated by IκB, a family of regulatory proteins. IκB binds to the p52 subunit of NF-κB, which limits the complex from gaining its entry to the nucleus. NF-κB activation is tightly regulated by TRAIL-mediated signals that degrade IκB. In breast cancer cells, these signals include ligand binding to DRs. Such signals activate a multicomponent IκB kinase complex that consequently phosphorylates IκB. Phosphorylated IκB undergoes proteasomal degradation, which enables free NF-κB to shuttle into the nucleus, bind to promoter, and activate transcription of proapoptotic genes.

It is notable that NF-κB activation antagonizes TRAIL-mediated apoptosis. TRAIL induces NF-κB pro-survival pathways by initiating cytosolic IκBα degradation and p65 nuclear translocation. A seleno-organic compound with glutathione peroxidase–mimetic efficacy, it predisposes TRAIL-resistant human breast cancer cells and xenograft tumors to apoptosis. 2-Selenium-bridged β-cyclodextrin triggers the expression of TRAIL receptors DR5 on both mRNA and protein levels in a dose-dependent mode and simultaneously inhibits NF-κB (Lin et al. 2011a, b). Via a similar approach, it was found that 2-tellurium-bridged β-cyclodextrin is a synthetic organotellurium compound significantly up-regulated DR5 and concomitantly repressed TRAIL-induced NF-κB prosurvival pathways (Lin et al. 2011a, b).

In addition, various proteins are necessary for TRAIL-mediated apoptosis because inhibition of these proteins results in attenuation of TRAIL-mediated responses. For example, MCF-7-MDR cells deficient in c-Myc display reduced TRAIL-induced apoptosis (Kim et al. 2011a, b).

Maximum growth and survival of ER-α-positive breast cancer cells require various proteins that are enhanced by estrogen. Investigation of both ER-α-positive and

ER-α-negative cell lines indicated that the effects of Sin3A were cell-type specific; Sin3A expression promoted growth of only the ER-α-positive cells. Sin3A down-regulated the expression of TRAIL, TRAIL-R1, caspase-10, and Apaf-1 in ER-α-positive cells. Therefore, technological approaches will be helpful in identifying practical obstacles that stand in the way of realizing genome-driven medicine with reference to ER-α-positive and ER-α-negative cancer (Ellison-Zelski and Alarid 2010).

Adenine nucleotide translocase-2 (ANT2) is overexpressed in breast cancer cells, which suppresses DRs and stimulates the expression of decoy receptors. In addition, it inhibits the transcriptional activity of p53 by preventing its phosphorylation by JNK at Thr81. An in vivo set of experiments using nude mice indicated that targeted inhibition of ANT2 caused TRAIL-resistant MCF-7 xenografts to undergo TRAIL-induced cell death, up-regulated DR4/DR5, and down-regulated DcR2 (Jang et al. 2010).

The WW domain containing E3 ubiquitin protein ligase 1 (WWP1) is an HECT (homologous to the E6-AP carboxyl terminus) domain-containing E3 ligase that is frequently amplified and overexpressed in ER-α-positive breast cancer. Pharmacological inhibition of WWP1 by siRNA induced apoptosis in breast cancer cells (Zhou et al. 2012).

The Smac mimetic SM-164 strongly enhances TRAIL activity by concurrently targeting XIAP and cIAP1 (Lu et al. 2011). The histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) triggered degradation of c-FLIP, thus resensitizing breast tumor cells to TRAIL (Yerbes and López-Rivas 2012). Doxorubicin or bortezomib combined with TRA-8, a monoclonal antibody to DR5, also suppressed Bcl-XL and XIAP in treated cells (Amm et al. 2011). Treatment with cisplatin–TRAIL also markedly repressed the expression of EGFR, p63, survivin, Bcl-2, and Bcl-xL in triple-negative breast cancer cells (Xu et al. 2011).

DDX3 was also identified as an important DR5-associated adaptor protein that evoked resistance against apoptosis at DR5 DD by recruiting inhibitor of apoptosis proteins to form DR5/DDX3/cIAP1 complexes. DDX3 with an absent CARD region would function as a naturally occurring dominant negative molecule and abrogate cIAP1 binding, resulting in high sensitivity to TRA-8-mediated apoptosis. The DDX3/cIAP1 complex is dominant over the DD complex, thus generating resistance to DR5-mediated apoptosis. To overcome this resistance, doxorubicin was provided, thus reducing DDX3 binding to DR5 in these cells and producing caspase activation and TRA-8 sensitization, suggesting that DR5/DDX3/cIAP1 complex regulation by doxorubicin reverses DDX3-mediated inhibition of TRA-8-induced apoptosis. Cell studies indicated that most basal cell lines expressed lower levels of DR5-associated DDX3 and cIAP1 than luminal and HER2-positive cell lines. TRA-8 inhibited growth of basal xenografts and produced 20 % complete 2LMP tumor regressions (Oliver et al. 2012). Further investigations were carried out and doubly enriched BrCSC ($CD44^+$, $CD24^-$, $ALDH^+$) cells were exposed to TRA-8 and examined for cytotoxicity, caspase activation, tumorsphere formation, and tumorigenicity. Doubly enriched BrCSC populations had cell surface expression of DR5 and were responsive to TRA-8-mediated cell death. In addition, TRA-8 at subnanomolar concentrations considerably inhibited 2LMP and SUM159 BrCSC tumorsphere formation (Londoño-Joshi et al. 2012).

Etoposide-stimulated ripoptosome formation converts proinflammatory cytokines into prodeath signals. Ripoptosome is a multicomponent cell death–inducing scaffold that contains the core components RIP1, FADD, and caspase-8, and it assembles in response to genotoxic stress-mediated depletion of XIAP, cIAP1, and cIAP2. It is negatively regulated by c-FLIP, cIAP1, cIAP2, and XIAP. Mechanistically, inhibitors of apoptosis proteins target constituents of this signalosome for ubiquitylation and inactivation (Tenev et al. 2011).

Lobaplatin displayed significant cytotoxic effects in p53-mutated triple-negative breast cancer. Cotreatment with TRAIL and platinum agents resulted in an augmented anticancer activity in triple-negative breast cancer cell lines (Engel et al. 2012).

A $CD44^+$ $CD24^{-/low}$ subpopulation of cells have CSC-like characteristics, together with pluripotency and a remarkable refractoriness to chemo- and radiotherapy. The cells exhibited enhanced surface expression of DRs and were more sensitive to both Fas- and TRAIL-mediated cell death pathways (Li et al. 2012).

Detailed mechanistic studies have indicated that human natural killer (NK) cells show antitumor efficacy against breast cancer cells. Liver NK cells expressed higher levels of TRAIL, and correspondingly, breast cancer cell lines expressed death-inducing TRAIL receptors, such as death receptor 4. Interleukin-2-stimulated liver and PB NK cells showed robust expression of CXCR3, which binds to the chemokines CXCL9, CXCL10, and CXCL11 that are secreted by breast cancer cells. Additionally, interferon-γ secreted from NK cells likely escalated the release of CXCL10 from breast cancer cells, which in turn promoted the migration of CXCR3-expressing NK cells into the tumor site (Kajitani et al. 2012).

Mounting evidence suggests that proteome changes are induced by overexpression of the p75 neurotrophin receptor (p75NTR) in breast cancer cells. Intriguingly, after treatment with TRAIL, fragments of cytokeratin-8, -18, and -19, as well as full-length cytokeratin-18, were stimulated robustly by p75NTR overexpression (Wilmet et al.

2011). Similar proteome differential distribution occurred in cell lines after treatment with doxorubicin and TRAIL (Leong et al. 2012).

MUC16 is a 20–25 MDa molecule with 22,152 amino acids in its protein sequence; various studies have highlighted its involvement in breast carcinogenesis. Emerging information has indicated that a polybasic amino acid sequence (RRRKK) in the cytoplasmic tail of MUC16 interacts with the ezrin/radixin/moesin (ERM) family of proteins. Janus kinases (JAKs) are nonreceptor tyrosine kinases, and their amino terminus domain harbors an ERM (4.1/ezrin/radixin/moesin) domain. MUC16 interaction with JAK2 in breast cancer cells was analyzed by reciprocal coimmunoprecipitation assay. It was observed that MUC16 coexisted with JAK2 in MDA-MB-231 cells, signifying that MUC16 interacted with JAK2 in breast cancer cells (possibly through the ERM domain). MDA-MB-231–ShMUC16 cells displayed a decrease in phosphorylated c-Jun and suppression of the proliferation of breast cancer cells by down-regulation of cyclin D1. There was an elevated interferon regulatory factor 1 mRNA level in the MDA-MB-231–ShMUC16 cells that triggered the expression of TRAIL (Lakshmanan et al. 2012). It is also essential to note that STAT proteins are considered to be involved in hampering TRAIL-mediated apoptosis. STAT proteins trigger the expression of antiapoptotic proteins (Mcl-1) in ER-α-positive cells. Bufadienolide compounds sensitize breast cancer cells to TRAIL-induced cell death via suppression of STAT3/Mcl-1 pathway (Dong et al. 2011).

TRAIL + Galectin

Investigational studies while working on endocytosis-deficient cells have indicated that cells display a marked increase in cell surface expression of galectin-3 (GAL3), an endogenous lectin, which colocalized with and coupled to DRs. Targeted inhibition of GAL3 effectively restored TRAIL sensitivity and enhanced TRAIL-mediated endocytosis of TRAIL/DR complexes (Mazurek et al. 2012). Intriguingly, in breast carcinogenesis, His64/Pro64 polymorphism of GAL3 is an important regulator in identifying cell responsiveness to TRAIL. Transfection of GAL3-deficient breast cancer cells with His64 GAL3 predisposed cells to TRAIL-mediated apoptosis, whereas Pro64 GAL3-transfected cells remained resistant to TRAIL (Amm and Buchsbaum 2011). Similarly, GAL3-enforced expression in GAL3-deficient breast cancer cells displayed differential responses to chemotherapeutic drugs. High levels of expression of His64 GAL3 resulted in induction of resistance in BT549 cells against doxorubicin, but cells expressing Pro64 GAL3 were resensitized to doxorubicin (Mazurek et al. 2011). Cell-type-specific studies have also suggested that GAL3 negatively regulated TRAIL-mediated apoptosis (Lin et al. 2009). Posttranslational modifications of proteins play a central role in determining the fate of proteins. In agreement, a recent study demonstrated that introducing phosphorylated GAL3 into GAL3-null, (TRAIL)-resistant human breast carcinoma cells promoted TRAIL-induced apoptotic cell death (Mazurek et al. 2007). GAL3 evoked resistance against TRAIL is a well-coordinated mechanism that involved downstream signaling. Akt is a major downstream PI3 K target reported to antagonize TRAIL-induced cell death. Cells overexpressing GAL3 cells displayed a high level of constitutively active Akt that greatly compromised TRAIL-mediated apoptosis; however, (PI3K) inhibitors blocked the GAL3 protective effect (Oka et al. 2005). In contrast to substantiating resistance against TRAIL, GAL3-enhanced, TRAIL-induced cytotoxicity through dephosphorylation of Akt via redox-dependent process (Lee et al. 2003).

TRAIL: Journey from Cell to Animal Model

The inherent complexity of genomic alterations in breast carcinogenesis, coupled with the numerous heterotypic interactions that occur between tumor and stromal cells, represents vital challenges in our quest to understand, interpret, and control metastatic disease. The incorporation of genomic and other system-level approaches, as well as technological breakthroughs in imaging and animal modeling, have galvanized the effort to overcome existing gaps in our understanding of breast carcinogenesis.

A growing body of evidence has suggested that administration of a recombinant adeno-associated virus (rAAV) vector expressing soluble TRAIL results in an efficient suppression of human tumor growth in nude mice. Various strategies are being evaluated by establishing breast cancer xenograft animal models and administering recombinant virus to evaluate the tumoricidal activity. Additionally, the gene expression system into the rAAV vector to control the soluble TRAIL expression is being monitored to determine the expression level of TRAIL for an effective apoptotic response (Liu et al. 2012a, b).

It is also important to note that CSCs are resistant to radio- and chemotherapy and play a significant role in breast cancer recurrence and metastatic disease. It is thus essential to find alternative strategies, such as immunotherapies that can be used to counteract this refractory subpopulation. Mammary CSC-like cells respond differentially to wide-ranging therapeutic interventions. Importantly, the $CD44^{+}$ $CD24^{-/low}$ subpopulation of cells within the transgenic mouse–derived AT-3 mammary carcinoma cell line was identified, which had CSC-like characteristics,

together with pluripotency and a prominent refractoriness to chemo- and radiotherapy. However, $CD44^{+}$ $CD24^{-/low}$ subpopulation of cells were more responsive to both Fas- and TRAIL-mediated apoptosis (Li et al. 2012).

For a better understanding of appropriateness of DR4 and DR5 for targeted therapy, various metastatic triple-negative cancer cell lines were treated with human agonistic monoclonal antibodies targeting TRAIL-R1 (mapatumumab) or TRAIL-R2 (lexatumumab). Lexatumumab was more useful than mapatumumab in activating caspase-8, inducing apoptosis and inhibiting long-term survival of metastatic cancer cells, which had cell surface expression of both TRAIL-R1 and TRAIL-R2. Contrary to this, human mammary epithelial cells transformed by oncogenic Ras were more sensitive to lexatumumab than nontransformed cells (Malin et al. 2011).

It is now evident that TRAIL-mediated apoptosis is antagonized by variety of proteins that need to be targeted to overcome resistance. In line with this approach, histone deacetylase is an attractive target. Cotreatment of BALB/c nude mice implanted with TRAIL-resistant invasive breast cancer MDA-MB-468 cells with HDAC inhibitor MS-275 and TRAIL reversed epithelial–mesenchymal transition, and inhibited tumorigenesis, angiogenesis, and metastasis (Srivastava et al. 2010)

With the bone microenvironment in metastatic conditions in mind, it was investigated whether osteoprotegerin (OPG) inhibited the activity of the TRAIL, raising the possibility that the anticancer activity of TRAIL might be compromised in the bone microenvironment where OPG expression was high. Experiments were conducted in a murine model of breast cancer growth in bone to assess the efficacy of TRAIL against intratibial tumors that were engineered to overexpress native full-length human OPG. It was observed that regardless of the secretion of supraphysiologic levels of OPG, treatment with Apo2L/TRAIL resulted in substantial growth inhibition of both empty vector and OPG-overexpressing intratibial tumors (Zinonos et al. 2011). Mounting evidence suggests that in vivo utility of TRAIL is limited by a short half-life in plasma due to a rapid clearance by the kidney. However, it has recently been demonstrated that these limitations can be overcome by using stably transduced adipose-derived mesenchymal stromal/stem cells (AD-MSC), which could serve as a constant source of TRAIL production. AD-MSC armed with TRAIL targeted a wide-ranging tumor cell line as well as murine models for breast cancer (Grisendi et al. 2010). Sophisticated information revealed that a multipronged approach may be an effective strategy. Bortezomib (Velcade), a proteasome inhibitor, is widely being used in combination with TRAIL; however, its therapeutic efficacy is greatly increased with coadministration of a Toll-like receptor 9 agonist, CpG. The pronounced effect of a multitargeted approach including α-DR5, bortezomib, and CpG for the prevention/treatment of spontaneous mammary tumors was observed in BALB-neuT mice, with impressive antitumor activity (Lee et al. 2010).

Gradually increasing skeletal tumor burden in bone metastasis is also a major consequence of carcinogenesis. RANKL (receptor activator of nuclear factor κB ligand) inhibition effectively inhibits pathologic osteolysis triggered by breast adenocarcinoma MDA-MB-231 cells in animals with established tumors; it also enhances the ability of TRAIL to suppress the skeletal tumor burden in vivo (Holland et al. 2010). A significant reduction in total tumor burden has also been observed in mice treated with bisphosphonates such as zoledronic acid alone or in combination with DR5 agonists (Szafran et al. 2009).

Detailed investigation has demonstrated that effective cancer therapy must selectively target tumors with the least amount of systemic toxicity. Attempts are being made to better integrate biotechnology and oncology. Thus, a nonpathogenic *Salmonella typhimurium* was engineered to secrete murine TRAIL under the control of the prokaryotic radiation-inducible RecA promoter. Systemic injection of *Salmonella* and induction of TRAIL expression with 2 Gy gamma irradiation caused a considerable decline in mammary tumorigenesis and reduced the risk of death by 76 % when compared with irradiated controls (Ganai et al. 2009).

Histone deacetylase inhibitors (HDACi) have gained much attention and have moved relatively quickly within the past few years toward clinical trials. Studies on HDACi raise the intriguing possibility that they play functional roles that are more significant than initially anticipated, which may provide opportunities to study the mechanisms of making cells responsive to TRAIL-mediated apoptosis. BALB/c nude mice were orthotopically implanted with TRAIL-resistant MDA-MB-468 cells and treated intravenously with an HDACi SAHA, TRAIL, or SAHA followed by TRAIL. SAHA counteracted growth of MDA-MB-468 xenografts in nude mice by suppressing tumor markers of uncontrolled undifferentiated proliferation, angiogenesis, and metastasis, and induced apoptosis (Shankar et al. 2009).

It seems likely that from these multiple and diverse therapeutic opportunities, coadministration of effective drugs with TRAIL will provide a new means of overcoming resistance against TRAIL-mediated apoptosis.

Vitamins and Breast Cancer

HER2/neu is an oncogene that facilitates neoplastic transformation as a result of its capability to disseminate growth signals, thus exhibiting ligand-independent behavior. It is

overexpressed in 20–30 % of human breast cancers correlated with aggressive disease and has been effectively targeted with trastuzumab. Recent information has suggested that orally administered α-tocopheryloxyacetic acid (α-TEA), a novel ether derivative of α-tocopherol, significantly counteracts primary tumor growth and suppresses the incidence of lung metastases in both transplanted and spontaneous mouse models of breast cancer. Results derived from experimentations of α-TEA plus HER2/neu-specific antibody treatment on HER2/neu-expressing breast cancer cells in vitro and in a HER2/neu-positive human xenograft tumor model in vivo were examined. It was revealed that α-TEA plus anti-HER2/neu antibody has an augmented and remarkable cytotoxic activity against murine mammary tumor cells and human breast cancer cells, and that the antitumor effect of α-TEA is independent of HER2/neu grade. α-TEA kills both HER2/neu-positive and HER2/neu-negative breast cancer cells (Hahn et al. 2011). Detailed experimental studies have indicated that dendritic cells (DCs) may perhaps be used for cancer immunotherapy because of their ability to process and present antigens to T cells. DCs may also be used in stimulating immunoresponses, even though DCs have only exhibited minimal effectiveness against established tumors in mice and humans. In an effort to improve the efficacy of DCs, a mouse model was used: 6-week-old female BALB/c mice were injected subcutaneously with DC and supplemented with an oral tocotrienol-rich fraction (TRF) daily (DC + TRF) and DC pulsed with tumor lysate from 4T1 cells (DC + TL). Results verified that TRF in combination with DC pulsed with tumor lysate (DC + TL + TRF), injected subcutaneously, extensively inhibited the growth of 4T1 mammary tumor cells as compared to a control group (Hafid et al. 2010).

The effectiveness of sequential treatment with paclitaxel and α-TEA was investigated in a BALB/c syngeneic 66cl-4-GFP mammary cancer model. Both agents were formulated into liposomes and delivered by inhalation in an attempt to augment antitumor efficiency and reduce paclitaxel toxicity. This combination approach was far better at reducing lung and lymph node micrometastatic foci when compared to control and individual treatment groups. Studies calculating and evaluating the toxicity profile of α-TEA indicated that α-TEA formulated in liposomes and delivered by aerosol or gavage for 25 days did not cause toxicity (Latimer et al. 2009).

The effectiveness of dietary administration of α-TEA was evaluated in the clinically relevant MMTV-PyMT mouse model of spontaneous breast cancer, which more closely resembles human disease. The minimal effective dose of α-TEA was calculated in the transplantable 4T1 tumor model, and it was demonstrated that there was a dose-dependent decrease of primary tumor growth and a reduction in metastatic spread to the lung. In situ analysis of tumor tissue identified cell death as an imperative mechanism of α-TEA-mediated tumor suppression besides inhibition of tumor cellular proliferation (Hahn et al. 2009).

Recently a study revealed comparative analysis of two vitamin E analogs, the esterase-hydrolyzable α-tocopheryl succinate (α-TOS) and the nonhydrolyzable ether α-TEA for their effects on HER2-positive breast carcinomas using a breast tumor mouse model and breast cancer cell lines. Ultrasound imaging revealed that α-TEA suppressed breast carcinomas in the transgenic animals better than its ester counterpart (Dong et al. 2012).

A study highlighted nanoformulation of D-α-tocopheryl poly(ethylene glycol) (PEG) 1000 succinate-b-poly (ε-caprolactone-ran-glycolide) diblock copolymer for breast cancer therapy. The results indicated that the fluorescence TPGS-b-(PCL-ran-PGA) nanoparticles could be internalized by MCF-7 cells, and moreover, TPGS-b-(PCL-ran-PGA) nanoparticles achieved a greatly elevated level of cytotoxicity. A MCF-7 xenograft tumor model on SCID mice showed that docetaxel formulated in the TPGS-b-(PCL-ran-PGA) nanoparticles efficiently counteracted tumorigenesis (Huang et al. 2011).

A recent study compared the anticancer therapeutic effect of palmitoyl ascorbate liposomes (PAL) and free ascorbic acid. To evaluate efficacy, biodistribution of pegylated PEG-PAL formulation was investigated in female BALB/c mice bearing murine mammary carcinoma (4T1 cells). In vivo anticancer activity of PEG-PAL was compared with free ascorbic acid therapy in same model; PEG-PAL treatment was appreciably more effective than free ascorbic acid treatment in counteracting tumor growth (Sawant et al. 2012).

It had previously been shown via cell culture models that calcitriol acts as a selective aromatase modulator and inhibits estrogen synthesis and signal transduction cascades in breast cancer cells. Another study examined the effects of calcitriol in vivo on aromatase expression, estrogen signal transduction cascade, and tumor growth when used alone and in combination with aromatase inhibitors. The dose–response curves in immunocompromised mice bearing MCF-7 xenografts indicated that higher doses of calcitriol resulted in significant tumor inhibitory effects (∼50–70 % decrease in tumor volume). Calcitriol extensively repressed estrogen levels in the xenograft tumors and neighboring breast adipose tissue. In addition, calcitriol inhibited estrogen signal transduction by repressing tumor ER-α levels (Swami et al. 2011).

Propolis is a product of the honeybee, and caffeic acid phenethyl ester (CAPE) is an important medicinal ingredient of propolis. CAPE, in a concentration-dependent manner, inhibits MCF-7 (hormone receptor positive) and MDA-MB-231 (a model of triple-negative breast cancer

tumorigenesis), both in vitro and in vivo, without off-target effects on normal mammary cells and robustly influences gene and protein expression. Furthermore, it is notable that CAPE dose-dependently represses expression of VEGF (vascular endothelial growth factor) by MDA-MB-231 cells and formation of capillary-like tubes by endothelial cells, thus generating inhibitory effects on angiogenesis (Wu et al. 2011).

Recently a study outlined antimetastatic activity of doxorubicin loaded in a pH-sensitive mixed polymeric micelle in mice using a murine mammary carcinoma cell of 4T1, which is one of the most aggressive metastatic cancer cell lines. When the tumor reached 50–100 mm^3 in size, the mice were treated with doxorubicin. The mixed micelle formulation resulted in counteracting tumorigenesis and no apparent metastasis until 28 days; however, considerable metastasis to the lung and heart was observed on day 28, when the mice were treated with doxorubicin carried by folate-conjugated, pH-sensitive polymeric micelles (Gao et al. 2011).

A recent mouse study assessed the effects of vitamin D deficiency on tumorigenesis in an osteosclerotic model of intraskeletal breast cancer developed by intratibial injection of MCF-7 breast cancer cells. It was observed that treatment with 1,25-dihydroxyvitamin D_3 inhibited uncontrolled cellular growth and proliferation, and increased apoptosis. It was also noted in animal model studies that in vivo, osteosclerotic lesions developed faster and were larger at the end point in the tibiae of vitamin D–deficient mice compared to vitamin D–sufficient mice (Ooi et al. 2010a, b). Similar results were obtained in a study evaluating effect of vitamin D deficiency on the intraskeletal growth of the human breast cancer cell line MDA-MB-231-TxSA in a murine model of malignant bone lesions (Ooi et al. 2010a, b). It is becoming increasingly clear that 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$ or calcitriol], the hormonally active vitamin D metabolite, exhibits anticancer actions in models of breast cancer and prostate cancer. In immunocompromised mice bearing MCF-7 breast cancer xenografts that ingested a vitamin D_3–supplemented diet, a considerable tumor shrinkage was observed. Comparative analysis indicated that both treatments equivalently repressed PC-3 prostate cancer xenograft growth, although to a lesser extent than MCF-7 tumors (Swami et al. 2012).

Encouraging attempts are being made to screen the most active vitamin analogs. In line with this attempt, the following were categorized for anticancer actions: two naturally occurring forms of vitamin E, α-tocopherol (αT) and RRR-γ-tocopherol (γT); the synthetic form of vitamin E, all-racemic-α-tocopherol (all-rac-αT); and an effective antitumor analog of vitamin E, RRR-α-tocopherol ether-linked acetic acid analog (α-TEA). Data indicated that γT, all-rac-αT, and α-TEA, but not αT alone or in combination with γT, considerably decreased the tumor burden of human MDA-MB-231 cells in nude mice. Both γT and α-TEA demonstrated promising anticancer properties in vivo and in vitro, whereas all-rac-αT demonstrated promising anticancer properties in vivo only (Yu et al. 2009).

Recently, the efficacy of a dendrimer-based nanotherapy that contained folic acid as the targeting agent and methotrexate as the chemotherapeutic drug was evaluated in an animal model of the artificial heterogeneous xenograft tumor. Human epithelial cancer cell line (KB) and a human breast adenocarcinoma cell line (SK-BR-3) were cocultured and preferential cytotoxic activity examined. Findings indicated that the nanotherapy was preferentially cytotoxic to KB cells (Myc et al. 2010).

Results derived from the assessment of the chemopreventive efficacy of the antioxidant lipoic acid in mice overexpressing wild-type HER2/neu as an animal model of breast cancer and in APCmin mice for intestinal cancer indicated a cell-type-specific differential response. Findings revealed that lipoic acid doses that were chemopreventive in APCmin mice instead increased breast cancer growth in HER2/neu mice (Rossi et al. 2008).

A combination approach using epigallocatechin gallate, resveratrol, and γ-tocotrienol at suboptimal doses in ER-positive MCF-7 breast cancer cells as a model was explored. Results indicated that suboptimal dose (10 μM) of each phytochemical resulted in a significant additive effect in suppression of cell proliferation. Likewise, it was observed that the combination of resveratrol and γ-tocotrienol resulted in restricting cellular proliferation, whereas the three phytochemicals added together did not produce more pronounced inhibition of cell proliferation (Hsieh and Wu 2008).

Researchers' access to validated technology to develop models and to perform mechanism-based biomarker assays has provided the means to rapidly test the carcinogenicity and the preventive/therapeutic efficacy of novel pharmacological agents, naturally occurring vitamins, and phytochemicals.

TRAIL Nanoparticles

Nanotechnology has the potential to revolutionize cancer diagnosis and therapy. Advances in protein engineering and materials science have contributed to novel nanoscale targeting approaches that may prove useful in clinical management of cancer patients. With the advent of nanotechnology, arranging materials into specific, organized structures at the nanoscale has become increasingly important, and various therapeutic nanocarriers have

accordingly been approved for clinical use. However, there are only a few clinically approved nanocarriers that incorporate molecules to selectively bind and target cancer cells. Here we examine some of the formulations and discuss the challenges in translating basic research to the clinic. We detail the armamentarium of nanocarriers and molecules available for selective cancer cell targeting and apoptosis.

Research results have indicated that magnetofection of plasmids driven by tumor-specific hTERT promoters provide an efficient technique to deliver therapeutic genes. One example is of the plasmid pACTERT-TRAIL, which utilizes human telomerase reverse transcriptase promoter, a tumor-specific promoter, to drive TRAIL. To improve the TRAIL gene transfer, a Fe_3O_4-PEI–plasmid complex was generated; the iron oxide nanoparticles were modified by positive-charge polyethylenimine to enable them to carry the negatively charged plasmid to induce apoptosis (Miao et al. 2012). The biological half-life and antitumor activity of TRAIL was also enhanced by using PEG-exposed nanoparticles (Lim et al. 2011).

There are other approaches in which TRAIL is conjugated to polymeric ultrasound contrast agents (UCA; microencapsulated gas bubbles). These can be traced and identified by ultrasound imaging, as well as fragmented into nanoparticles by focused ultrasound. This tumor-targeted delivery method is more proficient than solid nanoparticles; further, cells treated with TRAIL-UCA also present considerable apoptosis compared to unmodified UCA (Wheatley et al. 2012). Additionally, dimerized HIV-1 TAT peptide and nanoparticle vector (dTAT NP) have also been designed to improve the efficiency of a cell-penetrating strategy for tumor-targeted gene delivery. Administration of dTAT NP–encapsulated TRAIL pDNA noticeably restricted tumor growth (Kawabata et al. 2012). Human serum albumin (HSA) nanoparticles surface-modified with TRAIL and transferrin, and containing doxorubicin were designed to evaluate the ability of killing tumor cells in various organ tissues. Data indicated that the TRAIL/transferrin/doxorubicin HSA nanoparticle had remarkable cytotoxic and apoptotic activities in all cancer cells with a general or a drug-resistant phenotype, and that these nanoparticle had observable synergistic cytotoxic effects (Bae et al. 2012). Various delivery systems, including the anticancer drug doxorubicin and the therapeutic gene pTRAIL-loaded host–guest codelivery system, possessed better in vivo retention of chemotherapeutic drugs and exhibited remarkable therapeutic effects in the inhibition of tumor growth (Fan et al. 2012). Similarly, cancer cells transfected with gene-carrying cationic nanoliposomes displayed substantially elevated apoptosis (Sun et al. 2012). Codelivery of TRAIL with chemotherapeutic drugs like doxorubicin and paclitaxel using micellar nanoparticles also displayed great potential in cancer therapy (Lee et al. 2011a, b).

Codelivery of therapeutic-gene-encoding pORF-hTRAIL and doxorubicin has been performed with a tumor-targeting carrier, peptide HAIYPRH (T7)-conjugated PEG-modified polyamidoamine dendrimer (PAMAM-PEG-T7). T7, a transferrin receptor–specific peptide, was selected as a ligand to direct the codelivery system to the transferrin receptors expressing cancer cells. This codelivery system also resulted in a potent antitumor effect (Han et al. 2011). TRAIL-loaded human serum albumin sustained-release nanoparticles have recently been designed that have resulted in an increased circulating half-life period, and tumor distribution of TRAIL-HSA nanoparticles was also enhanced at 1 h after injection (Kim et al. 2011a, b).

It was also investigated in BALB/c mice electrovaccinated with DNA-encoding wild-type human DR5 (phDR5) that in vivo, hDR5 reactive immune serum prevented growth of SUM159 triple-negative breast cancer cells in SCID mice (Piechocki et al. 2012).

Monoclonal agonistic antibody–mediated chemotherapeutic drug–loaded nanoparticles also offer an exciting avenue for targeting chemotherapy and immunotherapy to DR5-overexpressing metastatic cancers. Chemotherapeutic drug dacarbazine-loaded polylactic acid nanoparticles, which were covalently conjugated to a highly specific targeting functional TRAIL-receptor 2 (DR5) monoclonal antibody, exhibited increased in vitro anticancer activity (Ding et al. 2011).

TRAIL + Cytoskeleton

The cytoskeleton is a cellular network of structural, adaptor, and signaling molecules that regulates most cellular functions, including migration and invasion.

Cells treated with TRAIL and microtubule-targeting agents show increased degradation of spindle checkpoint proteins such as BubR1 and Bub1, with consequent impairment of the mitotic checkpoint and induction of cancer cell death. Treatment of T98G and HCT116 cells with nocodazole alone results in a minimal pro-apoptotic response. However, addition of TRAIL to either nocodazole or paclitaxel (Taxol) maximizes the pro-apoptotic response (Kim et al. 2008). TRAIL-mediated apoptosis in cells overexpressing Bcl-2 is suppressed as cytochrome c released from mitochondria is blocked by Bcl-2. Microtubule-targeting agents are documented to enhance TRAIL-mediated apoptosis in cells having overexpressed Bcl-2 via either Bcl-2 inactivation or with alteration of the cytochrome c release mechanism. The combination of these factors weakens the Bcl-2 cytoprotective effect in the case

of synchronized action of TRAIL and cytoskeleton inhibitors (Gasparian et al. 2008).

More significantly, targeting the cytoskeleton at the transcriptional level by using myocardin-related transcription factor (MRTF) depletion to interrupt the transcription of cytoskeletal proteins also causes TRAIL sensitization in MDA-MB-231 cells. MRTF-A and -B are transcriptional coactivators for serum response factor, a commonly expressed MADS-box transcription factor contributory to the transcription of many genes with cytoskeletal functions. MRTFs are sequestered by monomeric G-actin; however, when Rho signaling causes polymerization of G-actin to form F-actin, they translocate into the nucleus and trigger the expression of target genes. Results indicated that MRTF-deficient cells expressed significantly increased levels of surface DR5 (Phipps et al. 2011). Likewise, pyrrolo-1,5-benzoxazepine-15 (PBOX-15), a novel microtubule-targeting agent, suppresses antiapoptotic proteins, up-regulates DRs, and potentiates TRAIL-mediated apoptosis in cancer cells (Maginn et al. 2011). 6-Acetyl-9-(3,4,5-trimethoxybenzyl)-9H-pyrido [2,3-b]indole (HAC-Y6) is a microtubule inhibitor that enhances up-regulation of the DR4 and represses antiapoptotic proteins (Tsai et al. 2010). Jurkat cells treated with TRAIL displayed attenuated adhesion to human umbilical vein endothelial cells and laminin, as well as their transendothelial migration. This compromised tumor metastasis was primarily the result of decreased intracellular Ca^{2+} concentration and depolymerized actin (Jiang et al. 2006).

Cells treated with TRAIL undergo apoptosis, and metastasis is abrogated. However, in various situations, proteins of specific signal transduction cascades are hyperactivated, and these proteins stimulate metastasis—for example, CD95 ligand and TRAIL-stimulated invasion of colorectal tumor cells and liver metastases in a K-Ras-dependent manner. Interestingly, loss of mutant K-Ras switched CD95 and TRAIL receptors back into apoptosis mode and abrogated metastatic potential. Oncogenic K-Ras and its effector, Raf1, manipulate DRs into invasion-inducing receptors by repressing the Rho kinase (ROCK)/LIM kinase transduction pathway; this is essential for K-Ras/Raf1-driven metastasis formation. Also investigated was the notion that K-Ras and Raf1 suppressed ROCK/LIM kinase–mediated phosphorylation of the actin-severing protein cofilin. However, cells reconstituted for ROCK or LIM kinase allowed CD95L to induce apoptosis in K-Ras-proficient cells and prevented metastasis formation (Hoogwater et al. 2010). It is therefore important to identify proteins that predispose cells to metastasis after treatment with TRAIL. However, ROCK-mediated metastasis suppression is also context dependent, as caspase-8-mediated cleavage of ROCK-1 is a novel mechanism for acquiring amoeboid shape and enhanced invasiveness in response to TRAIL and CD95L in mutant PIK3CA-expressing cells (Ehrenschwender et al. 2010).

TRP Channels in Breast Cancer

Several lines of study have verified that the process of tumorigenesis involves the altered expression of one or more transient receptor potential (TRP) proteins, the details of which can be found elsewhere (Farooqi et al. 2011).

Importantly, TRPV6 expression is higher in invasive areas, compared to the corresponding noninvasive ones. Targeted inhibition of TRPV6 in MCF-7 and MDA-MB-231 cells resulted in suppression of migration and invasion (Dhennin-Duthille et al. 2011). A recent study indicated the presence of chemical inhibition of TRPV6 channels via synthesized molecules based on the lead compound TH-1177 (Landowski et al. 2011). It is now known that TRPV6 is triggered by estrogen and progesterone. Furthermore, breast cancer cells exposed to the ER antagonist tamoxifen displayed reduced expression of TRPV6 (Bolanz et al. 2008). Tamoxifen also exhibited inhibitory effects in ER-negative breast cancer cells. However, these inhibitory effects are antagonized as a result of hyperactive protein kinase C. Therefore, targeted inhibition of protein kinase C is necessary to recapitulate the inhibitory effects of tamoxifen (Bolanz et al. 2009). Protein kinase C epsilon has been documented to be involved in desensitizing cells to TRAIL-mediated apoptosis (Shankar et al. 2008). It is encouraging to note that ER-negative cells also undergo apoptosis after cotreatment with tamoxifen and TRAIL (Lagadec et al. 2008).

On a similar note, TRPM8 is overexpressed in breast adenocarcinomas; this is correlated with an ER-positive tumor status. Exposure of the breast cancer cell line MCF-7 to 17-β-estradiol resulted in up-regulation of TRPM8 (Chodon et al. 2010). Additionally, targeted inhibition of TRPM7 in MCF-7 cells resulted in suppression of breast cancer cell proliferation (Guilbert et al. 2009). It is imperative to explore whether TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid–activated actin remodeling. Knockdown of TRPV4 expression entirely abrogated ascorbic acid–mediated endothelial cell migration (Fiorio et al. 2012).

MCF-7 cells express transient receptor potential canonical 1 (TRPC1) channels, and targeted inhibition of TRPC1 resulted in suppression of uncontrolled cellular proliferation (El Hiani et al. 2009). TRPC6 mRNA and protein were greatly increased in breast carcinoma specimens in comparison to normal breast tissue; however, overexpression of TRPC6 protein levels was not associated with tumor grade, ER expression, or lymph node–positive tumors (Guilbert et al. 2008).

A recent study has indicated that abrogation of TRPM8 channels not only noticeably eliminated TRPM7 expression, but also increased the apoptosis of hepatic stellate cells induced by TRAIL (Liu et al. 2012a, b).

It is therefore important to explore the effects of targeted inhibition of TRP channels in cell lines and animal models, as well as the use of phytonutrients and vitamins to overcome the resistance against TRAIL.

Conclusion

We now understand that breast cancer arises from aberrant decision making by cells concerning their survival or death, proliferation or quiescence, and damage repair or bypass. These decisions are made by signal transduction cascades or networks that process information from both outside and within the breast cancer cell, initiating responses that determine the cell's survival and uncontrolled proliferation. The quest to interpret protein alterations in breast carcinogenesis has spanned well over half a century. The overwhelming number of proteins, coupled with the plethora of isoforms as well as disease heterogeneity, have been major challenges. Advancement in breast cancer proteomics has paralleled technological developments. Gradual progress in analytical techniques and the implementation of strategies to decomplex the proteome into manageable components have greatly facilitated the exploration of proteins across a wide dynamic range. Emerging data regarding TRAIL-mediated signaling that can presently be collected through proteomics permit the near-complete definition of breast cancer subproteomes, which reveals the alterations in cellular transduction pathways and developmental pathways. Simultaneously, exceptional challenges for treating metastases consist of their small size, high multiplicity, and dispersion to diverse organ environments. In this domain, nanoparticles have the capability to transport complex molecular cargoes to the major sites of metastasis; they can also target the cargo to specific cell populations within specific organs. Engineering sciences must be integrated with cancer biology and medicine to develop nanotechnology-based tools for treating metastatic disease.

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