

Two Diseases with One Hit: Inhibiting a Potential Diabetes Target to Reduce Cancer Risk and to Improve Anti-Cancer Therapy

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Abstract: Obesity is a well-recognized cancer risk factor. The increase in risk for colorectal, endometrial, breast and esophageal cancers associated with obesity ranges from 1.5- to as much as 3-fold. Obese patients develop more aggressive cancers that are less responsive to treatment. Here, we review the available data on an obesity-linked gene, SH2-domain-containing inositol 5-phosphatase-2 (SHIP2), in light of new experimental and clinical evidence of its pro-oncogenic role. A putative diabetes drug target, SHIP2 is an important negative regulator of insulin signaling that acts downstream of phosphoinositide 3-kinase (PI3-kinase). In mice, SHIP2 levels are increased by a high-fat diet, and its knockout prevents diet-induced obesity. Taking together these findings, we propose that SHIP2 is a potential anti-cancer target with a high therapeutic index owing to its cancer-specific overexpression and/or differential function combined with the absence of major untoward effects upon its loss of function in normal cells. We compare and contrast the pro-oncogenic function of SHIP2 with the current understanding of cancer-relevant functions of PTEN and PTP-1B, two negative regulators of insulin function. The provocative idea that a negative regulator of insulin function will positively influence oncogenesis presents the intriguing possibility that its inhibition will be a beneficial strategy for two major therapeutic areas: metabolic diseases (such as obesity and diabetes) and cancer.

Key Words: SHIP2, obesity, EGFR, estrogen receptor, PTEN, breast cancer.

INTRODUCTION

In spite of the notable progress in reducing cancer incidence and cancer-linked mortality, many new challenges continue to face cancer researchers, such as those posed by the obesity epidemic and the incidence of anti-cancer drug resistance. Therefore, research in new directions based on evidence-based hypotheses that challenge the prevalent thinking are clearly needed to develop better diagnosis and treatment strategies. To this end, this review focuses on new evidence that supports SHIP2 (also known as INPPL1 for inositol polyphosphate 5-phosphatase-like 1) as an unconventional drug target with dual indication, whose inhibition will benefit both obesity and consequent metabolic illnesses such as type 2 diabetes (T2D) as well as cancer.

Candidate drug targets in the phosphoinositide pathway - SHIP2 phosphoinositide phosphatase is a key component of insulin/phosphoinositide 3-kinase (PI3-kinase) signaling as it dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP3) generated by the insulin-activated PI3-kinase [1, 2]. Phosphoinositide (PI) lipids are critical second messengers in the cell signal transduction pathways triggered by insulin and other hormones, such as estrogen (via cytoplasmic signaling) and leptin, as well as by growth factors such as EGF, IGF and PDGF. PI lipids regulate glucose metabolism, cell survival/proliferation and cell adhesion/migration processes [3]. Deregulation of the PI3-kinase pathway underlies the development of both cancer and T2D; hence, several components

of the PI3-kinase pathway, including PI3-kinase itself, Akt and mTOR, are validated targets for anti-cancer and anti-diabetes drug development [4-6].

SHIP2 ROLE IN METABOLIC SIGNALING AND ENERGY HOMEOSTASIS

Structural and molecular basis for a novel SHIP2 function - Most reports on SHIP2 have been focused on insulin signaling, largely because tissues that are traditionally considered as insulin responsive, such as liver and skeletal muscle, show somewhat higher levels of mRNA for the otherwise ubiquitously expressed SHIP2 [1, 7]. Placenta and brain also express high SHIP2 mRNA levels, and these tissues are also considered insulin-responsive in a broader sense, since insulin-dependent glucose uptake and gene expression occur in the placenta [8, 9], while the insulin receptors of the brain play a critical role in the central regulation of glucose metabolism and fertility [10]. Although there is little information on the relative SHIP2 protein expression in various tissues, SHIP2 is originally thought to function as a negative regulator of insulin signaling based on the mRNA expression data, in a manner analogous to the structurally related gene *SHIP1* [7]. *SHIP1* expression is restricted primarily to hematopoietic tissues, where it plays an important negative regulatory role in immune receptor signaling. Because these two genes bear significant structural similarities and share substrate preferences, it is quite rational to deduce functional similarities between them. However, important differences exist between *SHIP1* and *SHIP2*, specifically in their protein interaction motifs, leading to differential protein interactions. In addition to an amino-terminal SH2 domain, both *SHIP1* and *SHIP2* possess a carboxyl-terminal proline-rich region and an NPXY motif (one in *SHIP2* but two in *SHIP1*). Also,

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there is a SAM (sterile alpha motif) domain carboxyl-terminus to the proline-rich region of SHIP2, but not in SHIP1. In hematopoietic cells, SHIP1 negatively regulates growth factor and antigen receptor signaling. The association of SHIP1 with the Grb2 adaptor protein is central for this function [11, 12]. In contrast, there is no evidence of a similar interaction between SHIP2 and Grb2. The divergent proline-rich region of SHIP2 uniquely interacts with filamin [13], c-Cbl-associated protein (CAP) [14], vinexin [15], intersectin [16] and c-Jun NH2-terminal kinase (JNK)-interacting protein 1 (JIP1) [17]. The unique presence of a SAM domain in SHIP2 suggests that the specific protein interactions mediated by this region may confer a function to SHIP2 different from that of SHIP1. Indeed, recent reports have identified Arap3 [18] and EphA2 [19] as proteins interacting with SHIP2 via the SAM domain. There is, therefore, a strong molecular basis for the possibility of functional divergence between SHIP1 and SHIP2.

A role in insulin signaling: Evidence from animal models – As mentioned earlier, by analogy to SHIP1, SHIP2 is thought to downregulate PI3-kinase signaling in non-hematopoietic tissues, such as liver, adipocytes, skeletal/cardiac muscle and brain, where SHIP2 mRNA is present at relatively higher levels than in other tissues [7, 20]. Early support for this theory came from gene knockout studies in mice. In 2001 Clement *et al.* reported that the targeted deletion of SHIP2 in mice led to neonatal lethality due to hypoglycemia caused by insulin hyper-sensitivity [21]. This apparent insulin-resisting function of SHIP2 is corroborated by the fact that diabetic mice (db/db strain) contain higher levels of SHIP2 in their muscles and fat than their normal counterparts [22]. Diabetic rats and humans are reported to contain mutations that may lead to increased levels of SHIP2 [23]. Many *in vitro* studies published soon after the mouse knockout article seemed to confirm the negative regulatory role of SHIP2 in insulin signaling with respect to glucose metabolism (detailed in next section). However, in a corrigendum published in 2004, Clement *et al.* reported that the SHIP2 knockout strain they generated had an inadvertent deletion of another gene, *Phox2a*, which raised the possibility that the observed phenotype of their SHIP2^{-/-} mice may not be an accurate and/or full representation of SHIP2 function [24]. Subsequently, another study showed that SHIP2 knockout in mice in fact causes only mild insulin hypersensitivity (*no lethality due to hypoglycemia*) as well as a pronounced resistance to high-fat-diet-induced obesity [25]. The resistance to high-fat-diet-induced obesity in SHIP2^{-/-} mice is suggested to be due to a shift toward enhanced energy expenditure. This can also be interpreted as a SHIP2 function required for the storage of energy as fat in adipocytes, although this notion remains untested. Importantly, homozygous SHIP2 null mice are developmentally normal and healthy in their adult life, indicating that pharmacological inhibition of SHIP2 is unlikely to produce serious side effects. Transgenic mouse studies involving the exogenous expression of wild-type or catalytically inactive SHIP2 also produced mild aberrations in insulin function [26, 27]. SHIP2 clearly is an important regulator of energy balance under normal physiological conditions, although the mechanisms regulated by this molecule are not clear at present [28]. Accordingly, SHIP2 is being pursued by both academic and industry experts as a candi-

date drug target for T2D treatment (for detailed reviews, see [2, 29-31]).

A role in insulin signaling: Evidence from cell models – Understanding SHIP2 function at the molecular and biochemical level has proved to be a bit more difficult than one would expect given its high structural similarities to the well-studied SHIP1 [1]. *In vitro* studies in adipocytes and muscle cells corroborated SHIP2-downregulated insulin signaling [32, 33]. As these studies used an adenoviral system for the ectopic overexpression of SHIP2, the effects observed could be largely exaggerated, if not totally artefactual. Even though tremendous overexpression of SHIP2 efficiently lowers the intracellular PIP3 level, its effect on early signaling events (Akt activation) are modest at best. Because the cellular uptake of adenoviral particles strongly activates the PI3-kinase/Akt pathway [34], the effect of SHIP2 on such an altered background could be misleading. In congruence with this line of reasoning, studies employing different expression vectors to exogenously express SHIP2 describe contradictory results with regard to Akt activation and cell cycle progression [34-37]. Recent RNA interference (RNAi) studies in adipocytes show no significant changes in insulin signaling in the absence of SHIP2 [38]. Interestingly, in a gene expression profiling study, SHIP2 depletion in C2C12 muscle cells produced no discernible effect on insulin-regulated gene expression [39]. These studies highlight the need for increased prudence when deducing the gene function based solely on exogenous gene overexpression studies. We propose a new mechanistic model, based on the emerging evidence for SHIP2's role in endocytic vesicular transport, to explain the function of SHIP2 in insulin action (please see Fig. 4 and the associated text).

While SHIP2 mRNA is ubiquitously expressed, SHIP2 protein levels in skeletal muscle and fat are increased with obesity or in response to a high-fat diet [22]. This highly intriguing observation defines SHIP2 as a part of the cellular response to a specific external environmental condition such as diet/energy overload. We are, at present, examining the generality of this phenomenon across various tissues and have evidence that this phenomenon occurs in mammary glands as well (N. Prasad and I. Camarillo, unpublished observations). The identification of upstream signals and the delineation of molecular mechanisms of SHIP2 upregulation in response to a high-fat diet and/or obesity will certainly lead to new and exciting information in this area of research.

EVIDENCE INDICATIVE OF A POSSIBLE ROLE FOR SHIP2 IN ONCOGENIC SIGNALING

A role in growth factor signaling – Most SHIP2 research has been focused on its role in insulin biology, while a few reports, including some from our group, described SHIP2 function in cells/tissues that are not considered insulin-responsive. Dr. Stuart Decker's group first showed that SHIP2 is involved in cell signaling pathways beyond that of insulin [40]. SHIP2 is tyrosine phosphorylated by mitogenic growth factors similar to insulin; however, SHIP2 associates with the Shc adaptor protein when treated with EGF and PDGF but not with insulin [40, 41]. This suggests a possible function for SHIP2 in growth factor signaling that is distinct from its role in insulin signaling. Furthermore, SHIP2 is

heavily tyrosine phosphorylated in Bcr-Abl transformed K562 leukemia cells, where it constitutively associates with Shc [42]. The significance of this in oncogenesis remains to be understood. Although many groups have reported in the past that tyrosine phosphorylation had no effect on SHIP2 catalytic activity, a recent report from Dr. Peter Downe's lab presents evidence contrary to this notion [43]. This study reports that the specific activity of tyrosine-phosphorylated SHIP2 located at the plasma membrane, upon treatment with the tyrosine phosphatase inhibitor vanadate or a cell-permeable vanadate derivative, is 5- to 10-fold higher than that of the non-phosphorylated enzyme. Clearly, mechanisms of the enzymatic activation of SHIP2, in addition to other possible regulatory mechanisms such as protein interactions and subcellular localization, must be taken into consideration to fully understand its function.

A role in cell adhesion pathways - We have observed that many cancer cell lines paradoxically express high levels of SHIP2 and reported that SHIP2 regulates cytoskeletal organization, adhesion and cell spreading via protein-protein interactions [44]. In this study we made use of HeLa cervical cancer cells where SHIP2 is expressed at high levels to report several original findings: a) cell attachment stimulates the tyrosine phosphorylation of SHIP2 and increases its association with p130^{Cas}, b) the transient expression of exogenous wild-type SHIP2 increases adhesion, c) SH2 domain-defective SHIP2 possessing the R47G mutation fails to promote adhesion (indicating the importance of the SHIP2-p130^{Cas} interaction) and d) the exogenous expression of a catalytic domain deletion mutant of SHIP2 (Δ RV) inhibits cell spreading. In a follow-up article we demonstrated that the adhesion and spreading of HeLa cells on type I collagen (a major extracellular matrix protein of the interstitium), but not on fibronectin, collagen IV, laminin or poly-L-lysine, induces the robust tyrosine phosphorylation of SHIP2 [45]. In this report we further showed that Src family kinases mediate the SHIP2 tyrosine phosphorylation induced by the attachment to a collagen-I-coated surface but not those induced by EGF or insulin. SHIP2 is a direct substrate of Src *in vitro*, and the tyrosines 986-987 forming the NPXY motif of SHIP2 are the major sites of phosphorylation for Src, both *in vitro* and *in vivo*. Phosphorylation on the NPXY motif induces SHIP2 association with the adapter protein Shc. This interaction occurs *in vivo* during cell spreading on collagen I in a Src activity-dependent manner. Disruption of this interaction with mutations in the NPXY motif causes deregulation of lamellipodia formation during spreading on collagen I [45]. Since the type of collagen as well as the expression pattern of its receptors (e.g., integrin α 2 β 1) regulates the behavior of motile cells, our results indicated that SHIP2 might be important for cell adhesion and motility during metastasis. Indeed, knockdown of endogenous SHIP2 in HeLa cells causes severe F-actin deformities (with focal contact structures replaced by actin spikes) and cell-spreading defects [46]. These morphologic changes are the result of the altered functioning of Rac1 and Cdc42—members of the Rho family of small G proteins.

A role in receptor endocytosis and degradation - In HeLa cells, SHIP2 silencing altered the distribution of early endocytic antigen 1 (EEA1)-positive endocytic vesicles and vesicles containing internalized EGF and transferrin. EGF

treatment of SHIP2 RNAi cells enhanced EGFR degradation, increased EGFR ubiquitination and increased the association of EGFR with c-Cbl ubiquitin ligase [46]. We further showed evidence for the constitutive, tyrosine phosphorylation-dependent interaction of SHIP2 with c-Cbl in HeLa cells [46] and in several breast cancer cell types [47]. Thus, these results demonstrate a novel role for SHIP2 in decreasing ligand-dependent endocytosis and downregulating EGFR in cancer cells [46]. These results also suggest that the altered genetic background of cancer cells may confer a distinct functionality to SHIP2, perhaps through tyrosine phosphorylation (which is detectable in unstimulated cancer cells but not in normal cells). We propose that this new function of SHIP2 is critical for cancer cell migration/invasion during metastasis and for cancer growth during tumorigenesis.

For the first time, our studies describe a novel role for SHIP2 in non-insulin-responsive tissues in pathways unrelated to insulin. In addition, they present some evidence for the possibility of a unique and distinct function for endogenously expressed SHIP2 in cancer cells. This may explain the apparent paradox of high expression levels for a suppressor of the oncogenic PI3-kinase pathway in cancer cells. Others have reported a role for SHIP2 in cytoskeleton remodeling, cell spreading and receptor endocytosis, while demonstrating alternate underlying molecular mechanisms [13, 15, 16, 18, 19]. Further corroborating evidence comes from a gene expression profiling study in C2C12 myotubes, in which the suppression of SHIP2 altered the expression of several genes involved in cytoskeleton function, including adducin-alpha, pallidin, stathmin-like-2 and synaptojanin-2 binding protein [39]. A SAM-domain-mediated interaction of SHIP2 with oncogenic EphA2 receptor is shown to be important for EphA2 endocytosis in MDA-MB-231 (MDA-231) breast cancer cells [19]. In addition, Koch *et al.* demonstrated that SHIP2 binds to c-Met directly in MDCK cells and is involved in hepatocyte growth factor (HGF)-induced lamellipodium formation, cell scattering and cell spreading [48]. Recently, SHIP2 has been shown to associate with intersectin 1, a major regulator of EGFR endocytosis [49], and recruit it to the plasma membrane in response to EGF treatment [16]. While SHIP2 function in these cancer-relevant processes seems to be well supported, the plethora of putative underlying molecular mechanisms highlights the nature of the task ahead in delineating the importance of each of these known and of other yet-to-be discovered pathways in any given type of cancer. Nonetheless, these results clearly herald a new direction in SHIP2 research, one that is different from the widely held notion that SHIP2 acts primarily to suppress insulin signaling. Taken together, mounting evidence suggests that SHIP2 could be an important mediator of oncogenic pathways stimulated by oncogenic receptors (e.g., EGFR) through altered endocytosis and actin remodeling. Such an action by SHIP2 in response to type I collagen-integrin ligation [45] may determine the ability of cancer cells to migrate and attach to collagen-rich environments and may promote cell proliferation in low growth factor concentrations.

Indeed, evidence in the literature supports the existence of crosstalk between insulin and EGF signaling. Insulin resistance and T2D are known to enhance EGF signaling [50, 51], and EGF mimics insulin activity under insulin resistance

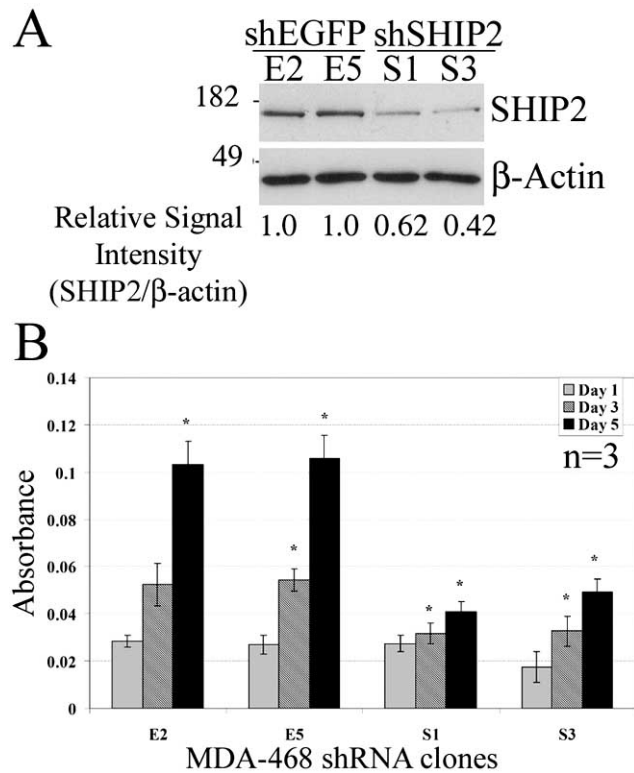


Fig. (1). SHIP2 suppression inhibits cell proliferation in MDA-468 breast cancer cells: Two stable clones expressing SHIP2shRNA and two control clones containing EGFP shRNA were analyzed in (A) western blot assays to demonstrate the extent of SHIP2 silencing, and (B) cell proliferation assays (carried out in triplicates, using a tetrazolium-compound based method). Asterisks on top of the bars indicate differences on Day 3 and Day 5 were significant ($P < 0.05/t$ -test) between clones S3 or S5 when compared to control E5, and were significant ($P < 0.05/t$ -test) when either of the SHIP2 clones were compared with E2 on Day 5 [53].

conditions [52]. SHIP2-mediated negative regulation of ligand-dependent internalization of EGFR by SHIP2, which consequently increases EGFR signaling, points to a possible role for SHIP2 in the above phenomenon. In light of recent data showing the activation of SHIP2 by tyrosine phosphorylation [43], we propose that SHIP2 function is altered upon specific tyrosine phosphorylation mediated by the activated EGFR or by type I collagen. The kinetics and downstream effects (e.g., interaction with Shc) of such phosphorylation are different from those induced by insulin and therefore suggest a unique and distinct function for SHIP2 in insulin-resistant/diabetic cells with upregulated EGF signaling or in cancer cells harboring an activated EGFR pathway.

EXPERIMENTAL EVIDENCE FOR A PROONCOGENIC ROLE OF SHIP2

We conducted experiments to test the hypothesis of a pro-oncogenic function for SHIP2 using *in vitro* and *in vivo* approaches [47]. We found that endogenously overexpressed SHIP2 in MDA-231 breast cancer cells promotes cell proliferation and that this effect is coupled to the EGFR expression level [47]. Similar results were obtained in MDA-468 breast cancer cells (Fig. (1)) [53]. In addition, SHIP2 func-

tion is important for *in vivo* cancer development (Fig. (2)) and spontaneous lung metastases in a nude mice mammary fat-pad xenograft model [47]. SHIP2 silencing in MDA-231 cells decreases the EGFR levels and enhances the cellular response to anti-EGFR drugs 3- to 5-fold, clearly indicating a major role for SHIP2 in determining the efficacy of anti-EGFR drugs [47]. Interestingly, SHIP2 regulates the endocytosis of other receptors as well, such as the EphA2 receptor in MDA-231 cells [19] and the PDGF receptor in pre-adipocytes [35]. A dominant negative version of SHIP2 inhibits PDGF-induced cell proliferation and suppresses ERK and Akt activation [35]. Recently we demonstrated that the SHIP2-mediated regulation of EGFR levels results in marked changes in downstream events in EGF signaling. In MDA-231 cells, SHIP2 silencing suppresses EGF-induced Akt activation and inhibits the expression of CXCR4, a major downstream gene of the EGFR- PI3-kinase pathway [54]. Furthermore, we reported for the first time that SHIP2 knockdown substantially reduces the spontaneous and EGF-induced migration of MDA-231 cells in wound closure assays [54].

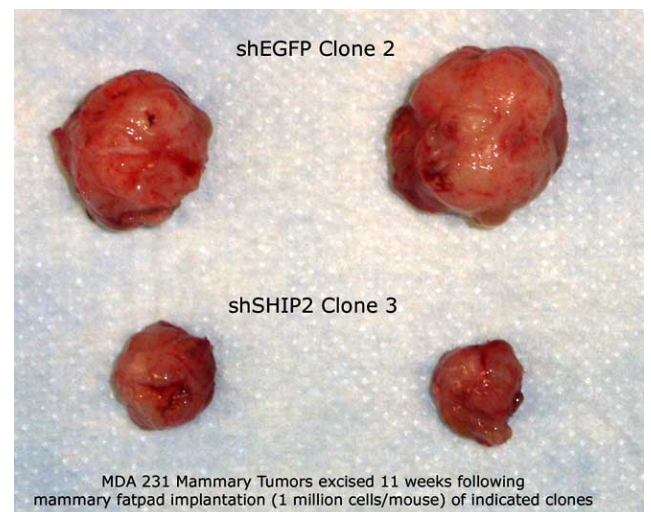


Fig. (2). Endogenous SHIP2 supports *in vivo* breast cancer development: 10^6 cells of control shEGFP clone or shSHIP2 clone of MDA-MB-231 mammary carcinoma cells grown for 11-weeks subsequent to implantation in the mammary fat pads of 7-8 week old nude mice. SHIP2-silenced cells showed 3-week growth delay (at 750 mg evaluation size) and had a dramatically decreased extent (while moderately decreasing the incidence) of spontaneous lung metastases [47].

Other groups have previously reported an association between the enhanced expression of SHIP2 and the proliferative phenotype in thyrocytes [7] and proliferating neurospheres [55]. Interestingly, EGF treatment increased SHIP2 mRNA expression in thyrocytes, and high SHIP2 protein expression coincides with high EGFR expression in neurospheres. On the other hand, an anti-proliferative function for SHIP2 has been reported in U87-MG glioblastoma cells [34], K562 erythroleukemia cells [37] and INS1E insulinoma cells [56]. All of these studies employed an adenovirus-mediated exogenous expression technique, therefore, as in studies with insulin function (explained in the previous section), these

results may not accurately represent the function of endogenous SHIP2 expressed in cancer cells.

CLINICAL EVIDENCE FOR A PRO-ONCOGENIC ROLE OF SHIP2

SHIP2 is overexpressed in breast and endometrial cancers – We carried out a large-scale immunohistochemistry-based analysis of SHIP2 expression in normal and cancerous breast tissue from 285 patients [57]. SHIP2 expression was scored in 233 cancer tissues and 227 adjacent normal tissues ($n = 460$). We first determined the median expression level in the pool containing normal and cancer tissues, followed by the stratification of the specimens as SHIP2-low (\leq median expression score) or SHIP2-high ($>$ median expression score) expressers. Based on this scale, SHIP2 is expressed at high levels in nearly half of all cancer tissues, while only 1 in 7 normal tissues showed high SHIP2 expression. We also recorded a similar high level of SHIP2 expression in endometrial cancers [58]; interestingly, nearly 60% of U.S. cases of endometrial cancers are linked to obesity [59].

The association of SHIP2 expression with aggressive tumor behavior – Our immunohistochemical study of primary breast cancers also provided evidence for a strong association between SHIP2 and aggressiveness of the disease [57]. The probability of SHIP2 overexpression (i.e., proportion of high SHIP2-expressing cancer tissues \div proportion of high SHIP2-expressing normal tissues; also called the risk ratio) was significantly higher in patients diagnosed at a younger age (≤ 50 yr; risk ratio = 4.13) as compared to those diagnosed later in life (> 50 yr; risk ratio = 2.37), as well as in aggressive invasive carcinomas (risk ratio = 3.52) when compared to non-invasive, ductal carcinoma in situ (DCIS) (risk ratio = 2.22). Invasive carcinomas diagnosed at a younger age (≤ 50 yr) showed the highest probability for SHIP2 overexpression (risk ratio = 4.38). Thus, clinical evidence indicated that SHIP2 overexpression is more likely to be associated with early-onset as well as the invasive type of the disease. Thus, SHIP2 expression signals a worse form of the disease, raising the possibility that SHIP2 expression analysis could be a useful biomarker for prognosis. Indeed, our analysis showed that SHIP2 expression in invasive carcinomas correlated with shorter disease-free survival (DFS—time from treatment-induced remission to recurrence) and overall survival (time from diagnosis to cancer-related death). The median DFS in patients with SHIP2 overexpression was 2 yrs shorter than in those with SHIP2 at a normal level. Nearly two-thirds of the cases with shorter DFS (≤ 2 yrs) expressed high SHIP2, in stark contrast to the cases with longer DFS (> 7 yrs) where two-thirds of the cases expressed low SHIP2 [57]. These were all statistically significant differences that support the notion that SHIP2 is a valuable prognostic marker.

The fact that SHIP2 expression is significantly elevated in 38% of early stage, non-invasive DCIS cases also suggests that SHIP2 upregulation could be an early diagnostic marker. On the other hand, SHIP2 levels of normal cells positively correlated with that of cancer cells in DCIS (Spearman correlation coefficient; $\rho = 0.2342/P = 0.038$;) as well as in invasive carcinomas ($\rho = 0.2385/P = 0.0229$; Spearman) (N. K. Prasad and S. Bose, unpublished observations [57]). The

paracrine regulation of SHIP2 levels between cancer and normal cells, if confirmed, could be one explanation for this observation. High SHIP2 levels in normal cells (in the absence of morphologically detectable cancer), on the other hand, could signal a pre-malignancy. Thus, it is likely that detection of SHIP2 overexpression in normal cells would be an early indication of the impending development of breast cancer, whereas SHIP2 overexpression in cancer cells could predict the development of a more aggressive/invasive cancer. Given that SHIP2 levels could be induced by a high-fat diet or obesity, high levels of SHIP2 in morphologically normal cells might be indicative of an underlying oncogenic process induced by these perturbed metabolic signals. As a diagnostic test, SHIP2 overexpression for all breast cancers (both invasive and DCIS) showed a specificity of 0.86 and a sensitivity of 0.45 when the SHIP2 expression for tumor-adjacent normal tissues was used as the false positive and that for cancerous cells as the true positive. The sensitivity is greater in invasive carcinomas, reaching 0.51 with a specificity of 0.85. When SHIP2 levels in normal healthy individuals (instead of normal-looking tissues adjacent to cancer) are taken as the false positives (none of the 20 such specimens showed high SHIP2 expression [47]), the SHIP2 overexpression test becomes highly specific. These data therefore provide a basis for exploring SHIP2 as a prognostic marker and for developing parameters for a clinically valuable test of SHIP2 expression for diagnostic and/or screening purposes.

The relationship between SHIP2 and cancer relevant molecular markers - In invasive carcinomas, SHIP2 significantly correlates with EGFR presence and with an estrogen receptor (ER)-absent phenotype. However, in pure DCIS and in cancers with minimal invasion, SHIP2 coexpresses with ER at a moderately significant level [57]. Thus, SHIP2 is strategically expressed in the presence of ER in early, non-invasive stages and in the presence of EGFR in late, invasive stages of the disease, enabling it to switch on a crosstalk between ER-EGFR. We have also addressed whether there is a possible relationship between SHIP2 expression and that of another inositol phosphatase, the tumor suppressor PTEN. Loss of PTEN is a common event in many types of cancer, but the fact that 85-90% of breast cancers express PTEN led to the delineation of alternate mechanisms that explain the subversion of PTEN's tumor-suppressor function [60]. Interestingly, SHIP2 is thought to assume the PTEN-like negative regulatory function upon PTEN loss [61]. SHIP2 and PTEN expression in the primary cancer specimens and cancer cell lines we examined show no significant association [57]. While SHIP2 association with ER loss and EGFR presence is significant in the presence of PTEN, the relationship of SHIP2 with survival periods is significantly strengthened in the absence of PTEN. This indicates that a potential molecular network between SHIP2, ER and EGFR is not affected by PTEN expression. More importantly, these data do not support the notion that SHIP2 might substitute for the lost PTEN function. If this were true, one would expect to have reduced disease severity in SHIP2-high/PTEN-negative cases compared to SHIP2-low/PTEN-negative cases. However, PTEN seems to dampen the pro-oncogenic effects of SHIP2, indicating a functional competition between these two inositol phosphatases in the oncogenic process, in such a way that the

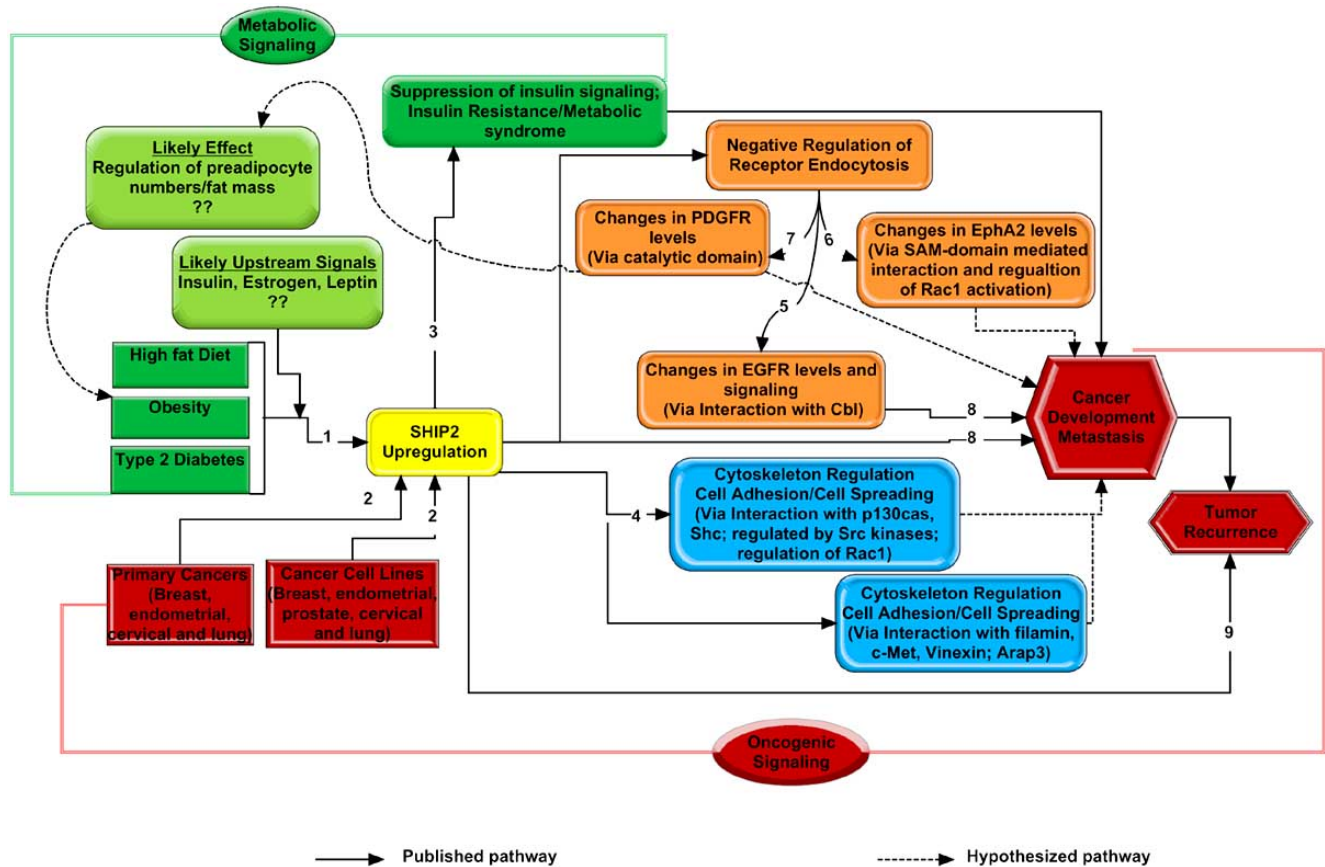


Fig. (3). A schematic overview of the current understanding of SHIP2 function: Experimentally elucidated role for SHIP2 in metabolic as well as oncogenic signaling illustrates several potential crosstalk points of regulation. The numbers inserted on a pathway denotes the respective citations as follows; 1) [22, 23]; 2) [47, 57, 58]; 3) [25-33]; 4) [44-46]; 5) [46]; 6) [19]; 7) [35], 8) [47]; and 9) [57]. Dotted lines represent hypothesized possibilities.

dominance of PTEN would be inhibitory while the dominance of SHIP2 would be stimulatory in effect.

Only one other report examines the levels of SHIP2 in primary cancer tissues. In hepatocellular carcinomas, SHIP2 expression in the cancer cells is decreased as compared to the adjacent normal cells present in the same cancer specimen ($n = 20$) [62]. This study focused on a putative relationship between glucose intolerance and survival. The results would have been more amenable for interpretation with a larger sample size and if normal healthy tissues were used as controls for the analysis of SHIP2 expression. Also, the study did not explore an association between SHIP2 levels and glucose intolerance or the aggressiveness of the disease. Nonetheless, SHIP2 in insulin-responsive liver may be regulated as a function of insulin response, and it is possible that SHIP2 might not have undergone the pro-oncogenic type of regulation that may occur in non-insulin-responsive cells such as mammary or endometrial cells. Further experiments to determine the exact nature of a possible cell-type-specific regulation of SHIP2 function are needed.

As shown in Fig. (3), SHIP2 is uniquely linked to energy balance (e.g., high-fat diet/obesity), metabolic perturbations (e.g., insulin resistance, T2D) and oncogenesis (via its actions on EGFR).

MOLECULAR MECHANISM(S) OF SHIP2 OVEREXPRESSION AND SHIP2 FUNCTION: A NOVEL LINK BETWEEN DIET/OBESITY AND CANCER

Molecular basis of Obesity-linked Cancer Risk - There is gathering epidemiological evidence for an increased cancer risk associated with obesity [59, 63, 64]. Given that our insightful data are largely from breast cancer studies, we will attempt to understand SHIP2 function with respect to that model. Obese postmenopausal women are ~50% more likely to develop breast cancers than lean women (for a detailed review see [59]). Breast cancers in obese individuals are also more likely to be resistant to conventional treatments, with consequential poor prognoses [65]. Obesity in children in the United States and other western countries is rising at an alarming rate, with nearly 1 in 5 children being obese in 2004 [66]. These children are at high risk for developing insulin resistance [67, 68], which is perceived to be a trigger for oncogenic events [59]. The prevalent notion on how obesity confers cancer susceptibility points to energy overload-induced insulin resistance (via negative feedback at the cellular level) in peripheral glucose-utilizing/storing tissues, such as skeletal muscle, adipose tissue or liver, as the trigger that predisposes to the development of T2D [69]. The undesirable mitogenic effects of higher levels of circulating insu-

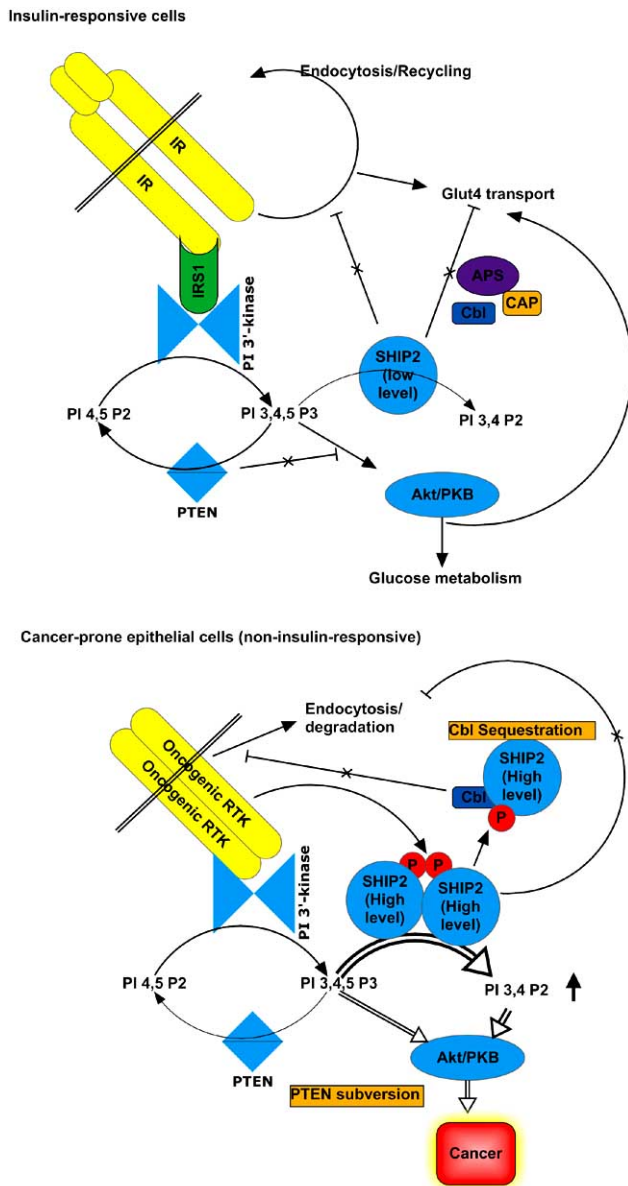


Fig. (4). A model for SHIP2 function: Here, we propose a model based on the concept of differential functioning of SHIP2 in normal insulin-responsive cells as opposed to cancerous non-insulin responsive cells. Some of the indicated relationships are hypothesized. For details, please see the associated text.

lin (as a consequence of hypersecretion of insulin by the pancreas to compensate for the peripheral insulin resistance) and estrogen (due to estrogen secretion by adipocytes) are thought to be responsible for the increased cancer susceptibility [59].

Anti-estrogen compounds or SERMs (selective estrogen receptor modifiers) have been available for clinical use for nearly three decades and have shown benefits in hormone-responsive breast cancers expressing the estrogen receptor (ER) [70]. However, the transformation of cancer from hormone-responsive (ER-positive) to hormone-resistant (ER-negative) status seems to be hastened by the use of SERMs (reviewed in [71]). Hormone-resistant cancers are often associated with enhanced expression of EGFR or its closely related homolog, HER2 [72, 73]. Acquisition of an activated

EGFR pathway is thought to be an important mechanism by which cancer cells turn estrogen-independent and consequently become resistant to SERMs. EGFR is an important drug target in breast cancer, as its inhibition has shown promising results in the clinic [74], even as the use of anti-EGFR drugs has produced many unpredictable outcomes due to new mutations in the target itself or in unrelated genes [75-77]. ER and EGFR constitute major molecular pathways of therapeutic importance to breast cancer [78, 79]; the inter-relationship between ER and EGFR, therefore, is of enormous clinical application. Estrogen can activate EGFR, and this non-genomic function of ER is important not only for breast cancer development but also for the development of normal female reproductive organs [80]. Anti-HER2 therapies can induce estrogen responsiveness in breast cancer cells [81]. Important intermediary molecules are GRP30 (a G-protein that can bind to estrogen and transactivate EGFR) [82], p130^{Cas} and Src [83]. While these provide important clues to the molecular basis of ER-EGFR crosstalk, they offer little to understand how these intermediates can become important at certain stage(s) of cancer progression to trigger the switch from ER-positive to ER-negative status. In addition, they do not address the issue of obesity-related cancer risk and aggressiveness. On the other hand, there is compelling evidence to propose SHIP2 as a unique and novel molecular link between high-fat diet/obesity/insulin resistance/estrogen and EGFR/cancer. It is perhaps more than mere coincidence that a SHIP2-interacting protein, p130^{Cas}, and an upstream regulator of SHIP2 function, Src kinase, are the key mediators of ER-EGFR crosstalk. We hypothesize that, based on the strong clinical and experimental data described in the previous section, SHIP2 is an inducible switch that elevates obesity-related cancer risk and confers drug resistance.

A Model for SHIP2 function – In Fig. (4) we outline a new model for SHIP2 function based on the notion that SHIP2 function is different or differentially regulated in a cell-type-dependent manner. In normal insulin-responsive tissues (e.g., liver, adipocytes and skeletal muscle), SHIP2 primarily regulates insulin function and energy metabolism. However, it is possible that more precise molecular mechanisms may yet be discovered and could likely involve more than just turning off PI3-kinase signaling at the phosphoinositide levels. We envision a possible scenario where SHIP2 primarily regulates vesicular transport in both normal and cancer cells. In insulin-responsive cells, the relevant target is likely to be the internalization and recycling of the insulin receptor. Alteration of this process by SHIP2 knockout would explain the observed phenotype of moderate insulin hypersensitivity in animal models, as insulin receptor internalization is closely coupled to IRS1 phosphorylation and subsequent Glut4 translocation [84, 85]. Glut4 translocation may also be regulated by SHIP2 via protein interactions with Cbl, CAP and APS, thereby impacting the TC-10-mediated process [14, 86].

Differential regulation of SHIP2 in cancer cells, most likely via the tyrosine phosphorylation induced by type I collagen, EGFR ligands or activated Src, is expected to support oncogenesis via two possible pathways: sequestration of Cbl and subversion of PTEN function. In the first pathway, by associating with Cbl in the cytoplasm and mediated by

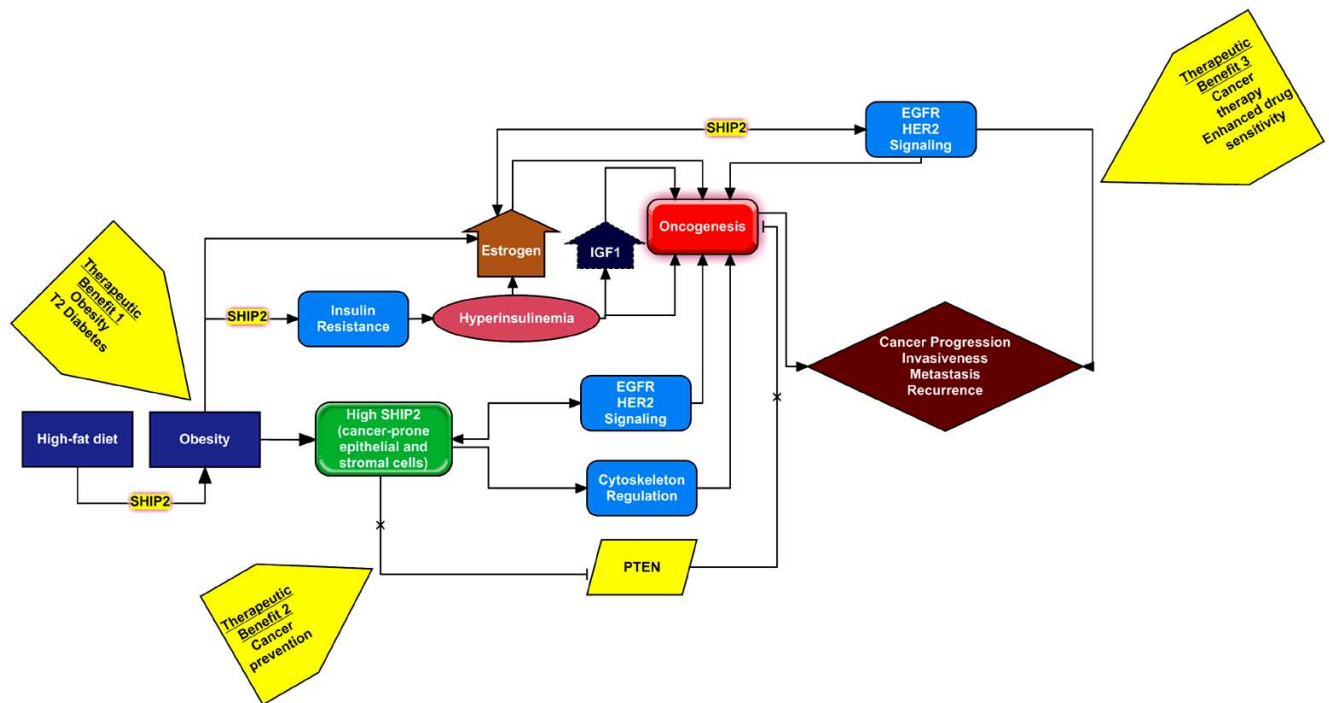


Fig. (5). Potential therapeutic benefits of targeting SHIP2: Specific inhibition of SHIP2 will; (a) prevent obesity and insulin resistance by improving energy homeostasis, (b) prevent cancer by interrupting the oncogenic consequences of obesity and (c) inhibit cancer progression/metastasis/recurrence by abrogating ER-EGFR crosstalk.

the tyrosine phosphorylation of SHIP2 by EGFR or collagen, SHIP2 will hinder EGFR-Cbl interaction, thereby inhibiting EGFR endocytosis and downregulation. EGFR- PI3-kinase -Akt activation will therefore be enhanced due to persistent signaling from the surface receptors. We suggest that this pathway is likely to affect many, if not all, oncogenic receptor tyrosine kinases (RTK), the biology of which may be determined by the cell type and the expression level of these RTKs. In the second pathway, SHIP2 is envisioned to successfully compete with PTEN for the available PIP3, thereby functionally overriding the negative effects of PTEN. The outcome would be higher levels of PI3,4P2, which is shown to be more stable and necessary for full activation of Akt [87, 88]. Thus, a more robust activation of the Akt pathway would ensue even in the presence of wild-type PTEN, a novel molecular mechanism of PTEN subversion. Thus, both pathways of SHIP2 action would lead to an enhanced PIP3-Akt pathway, promoting cancer cell proliferation and successful migration upon detachment from the basement membrane. It will be of great interest to analyze how the 4-phosphatases that dephosphorylate PI-3,4-P2 to suppress Akt activation and cell proliferation [89, 90] functionally interact with SHIP2 and PTEN in the oncogenesis process.

Our model predicts that the high expression of SHIP2 is a facilitating factor for cancer development and progression. Many vital links that support this model remain to be experimentally validated. For example, it remains to be seen whether the differential phosphorylation recorded in cancer cells could lead to the upregulation of SHIP2 expression. It is plausible that post-transcriptional regulation induced by external stimuli such as diet/environment or upon oncogenic transformation could increase SHIP2 levels, given the impor-

tance of the SHIP2 3'-untranslated region in message stability [23]. Biochemical and biological evidence for SHIP2 and PTEN competition for PIP3 is another aspect that is only beginning to be addressed [61].

FUTURE PERSPECTIVES

SHIP2 function is required for the conservation of energy in the form of adipose tissue [25]. Dietary regulation of SHIP2 protein levels [22] suggest that elevated SHIP2 may represent a metabolic response to high-fat diet/obesity. We propose that SHIP2 acts as a novel molecular link underlying the cancer risk posed by obesity and a high-fat diet [59] and that it supports oncogenesis via alteration in endocytic trafficking (e.g., via EGFR). The mechanisms regulated by SHIP2 hypothesized to cause obesity-related oncogenesis and/or aggressive disease outcomes (e.g., recurrence, drug resistance) are shown in Fig. (5). In this scheme the SHIP2-mediated regulation of oncogenic receptors such as EGFR [46, 47] represents a key molecular mechanism as disease advances to an invasive stage, leading to hormone independence and the development of aggressive, invasive disease with recurrence and mortality. As SHIP2 expression elevates, at a certain threshold level it may act as a switch from ER-positive to ER-negative status by ensuring higher levels of EGFR/HER2. SHIP2 overexpression is expected to negatively impact the cellular response to anti-ER and anti-EGFR drugs, as EGFR level/activation is linked to the degree of drug response [91, 92]. Clinical data support such a transition from ER-positive to ER-negative in the progression of breast cancers [93, 94]. To facilitate ER-EGFR crosstalk, SHIP2 must co-exist with ER. Indeed, SHIP2 overexpression in the presence of ER occurs in ~39% of DCIS and ~45% of

invasive carcinomas, highlighting the fact that SHIP2 overexpression and ER presence are not mutually exclusive events [57]. Upon disease progression to the invasive stage, a significant link between SHIP2 expression and both ER loss and EGFR presence becomes evident [57], supporting the idea that SHIP2-mediated upregulation of EGFR levels/signaling in effect may render the cells estrogen-independent.

There is general consensus on SHIP2 as a diabetes and/or obesity drug target [2, 29]. The idea presented here, on the other hand, is unconventional and among the first linking obesity to breast cancer. This provocative idea that a negative regulator of insulin function will positively influence oncogenesis is highly intriguing and novel. Another negative regulator of the PI3-kinase pathway, a phosphoinositid 3-phosphatase, is a classical tumor suppressor and a suppressor of insulin function (reviewed in [95]). Targeting PTEN for anti-diabetic function is hindered by the prospects of an oncogenic side effect [2]. However, a third well-studied negative regulator of insulin function, protein tyrosine phosphatase PTP1B, was recently shown to be required for HER2-induced mammary tumorigenesis [96, 97]. Therefore, it is clear that there is functional divergence among different negative regulators of insulin signaling, some of which could positively promote oncogenesis in the presence of a permissive signal and/or microenvironment. SHIP2, we suggest, will be a useful target with dual applications in both obesity/diabetes and cancer therapy (Fig. 5). More importantly, targeted SHIP2 inhibition could reduce and/or prevent breast cancer development as well as its poor disease outcome in obese and overweight individuals. In addition, SHIP2 inhibition will be useful in enhancing the therapeutic response of existing drugs targeting the EGFR family, ER and possibly others, including PDGFR and Bcr-Abl. We expect that further studies will validate SHIP2 as a clinically useful biomarker for cancer predisposition, early detection, drug resistance and relapse.

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