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Review

RLIP: An existential requirement for breast carcinogenesis

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ABSTRACT

Breast cancer (BC) is the most common cancer among women worldwide. Due to its complexity in nature, effective BC treatment can encounter many challenges. The human *RALBP1* gene encodes a 76-kDa splice variant protein, RLIP (ral-binding protein1, *RalBP1*), a stress-protective mercapturic acid pathway (MAP) transporter protein, that also plays a key role in regulating clathrin-dependent endocytosis (CDE) as a Ral effector. Growing evidence shows that targeting RLIP may be an effective strategy in cancer therapy, as RLIP is over-expressed in multiple cancers and is known to induce resistance to apoptosis and chemotherapeutic drugs. Recent studies demonstrated that RLIP is expressed in human BC tissues, as well as BC cell lines. Knockdown of RLIP resulted in apoptotic death of BC cells *in vitro*, and targeted inhibition and depletion of RLIP resulted in regression of BC in xenograft studies of nude mice. Signaling studies showed that RLIP depletion inhibited endocytosis and differentially regulated signaling to Akt, Myc, and ERK1/2. However, the proliferation and multi-specific transport mechanisms that promote RLIP-mediated cell death in BC are not well understood. In this review, we will discuss a missing but an essentially determining and connecting piece of the puzzle on the understanding of proliferation and transport mechanisms by focused analyses of the apoptotic, drug- and radiation-sensitivity regulated by RLIP, a stress-responsive non-ATP-binding cassette (ABC), high capacity MAP transporter, in breast cancer.

1. Introduction

Breast cancer (BC) is a heterogeneous malignancy that encompasses several distinct entities with remarkably different biological characteristics and clinical behavior, and remains one of the most prevalent diseases in the Western world, with approximately one in eight women predicted to be affected by BC in their lifetime. Improvements in detection, anti-estrogen therapies, and cytotoxic chemotherapy have led to increased survival rates from 72% in the 1980s to 89% in 2010. Despite these improvements, the incidence of BC has increased over the same period [1,2]. The American Cancer Society estimated that there would be approximately 266,120 new cases of invasive BC; 63,960 new cases of carcinoma *in situ* (CIS, non-invasive and the earliest form of BC); and 40,920 BC-related deaths in the United States in 2018. Approximately 90% of patient deaths are due to complications from recurrent or metastatic diseases. BC cells preferentially metastasize to specific organs, known as organotropic metastasis, depending on the subtype of BC, the host organ microenvironment, and cancer cells-organ interactions [3–5].

Effective and persistent treatment of BC is frequently hindered by the development of therapy resistance in BC cells, resulting from the inappropriate activation of a number of survival-promoting proteins, including extracellular signaling molecules (ligand) such as epidermal growth factor (EGF), transforming growth factor (TGF), or insulin-like growth factors (*e.g.*, IGF1). Binding of these ligands to their receptors activates a number of intracellular proteins (*e.g.*, Akt, Ras, NFκB) that induce the up-regulation of proteins necessary to resist stress and cell death. Receptor-ligand signaling is normally terminated through a process called endocytosis, in which the cell pinches off the patch of membrane containing the receptor-ligand pair, bringing it inside the cell into spherical membrane vesicles (bubbles) that have the ligand on the inside surface, and the receptor on the outside surface. The receptor and ligand can then be degraded in these vesicles, terminating signaling. However, endocytosis also appears to be necessary to activate

Abbreviations: ABC, ATP-binding cassette; BC, breast cancer; CDE, clathrin-dependent endocytosis; EGF, epidermal growth factor; ER, estrogen receptor; GSH, glutathione; GS-Es, glutathione-electrophile conjugates; IGF, insulin-like growth factor; MAP, mercapturic acid pathway; PR, progesterone receptor; RLIP, ral-interacting protein, *RalBP1*; TGF, transforming growth factor

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the signaling of other important ligands, such as Wnt, which plays an important role in the survival mechanism of BC cells. A common intersection point for signaling by EGF, TGF, IGF1, and Wnt is the activation of the potent survival protein Akt, which is frequently activated in BC and associated with poor survival. In addition to the activation of signaling mechanism that promote survival, BC cells rely on a number of small molecules for protection against cell death caused by poisonous chemicals, such as chemotherapy [6–10].

Glutathione (GSH) is a sulfur containing small molecule in cells that also protects cells from death caused by poisons. GSH chemically combines with poisons to make glutathione-electrophile conjugates (GS-Es), which are still toxic and must be removed from cells using energy-dependent pumps present in the cell membrane. We have shown that RLIP (ral-binding protein, encoded by the human gene *RalBP1*) of the mercapturic acid pathway (MAP) is the primary pump that removes poisons and their GS-Es from cells. This function of RLIP is more important in cancer cells than in normal cells; whereas depletion of RLIP does not kill normal cells, it is very effective in killing cancer cells [11]. For example, depleting RLIP causes significant BC cell death in culture and near complete regression of subcutaneous BC tumors established in nude mice [12–16]. Recent studies also suggest that 2′-hydroxyflavanone (2HF), a flavonoid present in citrus fruits, has great potential for the treatment of metastatic BC, and it can further potentiate the efficacy of RLIP depletion/inhibition *via* RLIP antisense and antibodies [4,5]. Studies summarized in this review provide strong evidence for the role of RLIP as an effector protein which catalyzes the efflux of GS-E, a process which is a crucial component of diverse cellular signaling pathways including mitosis, proliferation, differentiation, apoptosis, and endocytosis. Because a wide variety of cancer cells appear to rely more heavily on the anti-apoptotic effects of RLIP than non-malignant cells, and because of the striking efficacy of RLIP-depletion or inhibition in causing regression of solid tumors in animal models, it is an attractive target for development as an anti-neoplastic agent. In this review, we will also discuss the mechanisms underlying apoptosis caused by RLIP depletion/or inhibition and provide support for RLIP as an attractive therapeutic target in BC.

2. RLIP

In the last few years, extensive research has been made to elucidate the functional significance of RLIP. The resulting novel breakthroughs have helped us understand its transport and signaling functions. RLIP is a ubiquitously expressed, key stress-defensive, anti-apoptotic, multifunctional protein that transports GS-Es, thus controlling the intracellular concentration of pro-apoptotic oxidized lipid byproducts and other xenobiotics such as chemotherapeutic agents. These properties place RLIP at a very important position in the hierarchy of the stress defense mechanism adopted by the cell. Selective over-expression of RLIP in malignant cells of diverse origin is one of the possible mechanisms by which these cells overcome chemotherapy and radiation induced oxidative damage. RLIP (human gene *RalBP1*, ral-binding protein, 18p11.22), a major and multi-specific transporter of MAP, is a protein that is up-regulated in multiple cancers and is known to induce apoptotic and drug-resistance [8–11,17–32]. Recent publications have demonstrated the principal regulatory role of and extensive signaling cross-talk involving RLIP in multiple cancers [33–45]. Inhibition or depletion of RLIP cause tumor regression and has been used to elucidate the role of RLIP-liposomes in providing protection from radiation exposure and chemotherapy in BC [13–16,22–25,46]. RLIP is a crucial anti-apoptotic protein that is particularly important for the survival of certain cancer cells. It is over-expressed in many histological types of cancer. RLIP expression is significantly increased in the BC samples and positively correlated with the malignant status of BC patients [35]. The increased sensitivity of malignant cells compared to non-malignant cells, combined with a lack of animal toxicity at an effective dose, has led us to propose the development of RLIP antisense as a broad spectrum anti-neoplastic agent [11,15–19,27]. Furthermore, in recent studies, dramatic regression of established MCF7 (ER^{+ve}, PR^{+ve}, $Her2^{-ve}$) and SKBR3 (ER^{-ve}, PR^{-ve}, $Her2^{+ve}$) BC xenografts was observed in nude mice treated with RLIP antisense [15]. The mechanism of BC cell death involves activation of both intrinsic and extrinsic apoptosis pathways.

Recent studies have also shown that RLIP binds to p53 in the cell membrane, thereby potentially limiting the apoptotic response initiated by the nuclear translocation of p53 [26]. RLIP is a *rac* effector and has been shown to be an essential factor in determining the metastatic aggressiveness of cancers [37]. The transport of GS-Es by RLIP regulates the cellular response to both chemotherapeutic drugs and radiotherapy. The GS-Es of many administered chemotherapy drugs and toxic end-products of lipid peroxidation, like 4-hydroxynonenal (GS-HNE) generated by irradiation, are actively transported out of the cells by RLIP, thereby limiting the efficacy of either modes of clinical interventions [6,9]. Importantly, RLIP has been shown to be an essential prerequisite for epithelial chemical carcinogenesis [21].

RLIP is a crucial GS-E transporter frequently over-expressed in cancer cells, and is strongly linked with resistance to the apoptotic effects of a wide variety of chemical, as well as radiant stresses. There are no close structural RLIP homologs. ATP-binding cassette (ABC) transporter proteins are partial functional homologs, but together represent < 1/3 of the total transporter function, with RLIP representing the remainder [10,47,48]. Thus, the MAP for metabolism of endogenous and exogenous electrophilic toxins is almost completely blocked upon depletion of RLIP. These studies indicate that the accumulation of toxic pro-apoptotic metabolites triggers cell death preferentially in malignant cells, including melanoma, non-small-cell lung carcinoma, small cell lung carcinoma, kidney, prostate, colon, pancreas, neuroblastoma, and BC. The drug resistance phenotype in this wide variety of cancer cell types parallels the RLIP protein content of these cell types [9–12]. Non-malignant cells, which do not rely as heavily on MAP as an anti-apoptotic defense mechanism, are spared.

RLIP is also an important signaling interface between cancer and metabolic syndrome. Our recent studies have revealed that homozygous RLIP knockout ($RLIP^{-/-}$) mice have lower blood glucose levels than wild type mice and that RLIP is a principal determinant of the response to major metabolic syndrome drugs like atorvastatin, gemfibrozil, metformin and rosiglitazone [49]. These connections to metabolic syndrome have important implications for BC, as there is a direct and statistically significant epidemiological correlation between metabolic syndrome and the incidence of aggressive triple-negative BC (characterized by tumors that do not express estrogen receptor [ER], progesterone receptor [PR], or HER2). The term triple-negative BC encompasses a heterogeneous group of tumors that show distinctive, but rather heterogeneous, pathological and clinical features [50]. Thus, given the immense influence of RLIP on multiple critical factors that enhance the risk of BC (*e.g.*, those that favor proliferation and induce drug- and radio-resistance); RLIP represents a premier target to develop effective therapeutic treatments for aggressive breast tumors. Cancer cells are significantly more sensitive than normal cells to apoptosis triggered by blocking RLIP, suggesting the feasibility of targeting RLIP in BC therapy [11,15–20].

3. p53 (TP53)

Genomic data from > 20,000 cancer genomes provide a wealth of information on cancer gene alterations and have confirmed TP53 as the most commonly mutated gene in human cancer. Tumor protein p53, encoded by the human gene *TP53* [17p1.33], plays an important role in cancer prevention. Under normal conditions, p53 is maintained at a low level. However, in response to various cellular stresses, p53 is stabilized and activated, which, in turn, initiates DNA repair, cell-cycle arrest, growth, survival, senescence, autophagy, and apoptosis. Post-translational modifications of p53 including phosphorylation, ubiquitination,

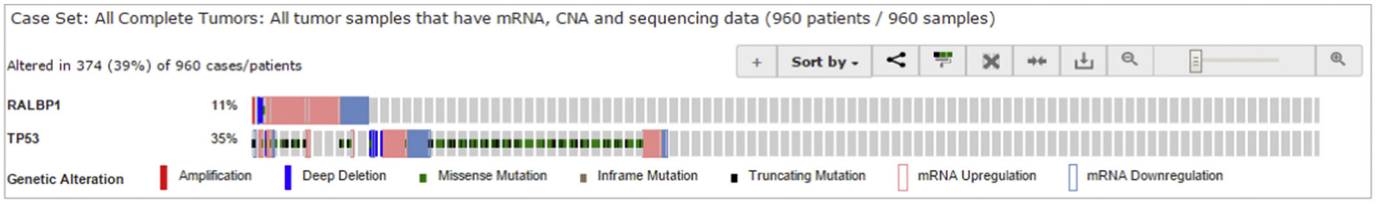

Fig. 1. C-bioportal analysis of the TCGA provisional breast cancer database: We queried the TCGA provisional database for copy number amplifications (CNAs) and mutations in RLIP (*RALBP1*) and p53 in BC. Results show a high (35%) rate of genetic alterations in p53 and an 11% in RLIP, with statistically significant co-occurrence ($p < .02$).

and acetylation at multiple sites are important to regulate its activation and subsequent transcriptional gene expression. Particularly, phosphorylation of p53 plays a critical role in modulating its activation to induce apoptosis in cancer cells. p53 known as the "Guardian of the Genome," functions as a stress-responsive, genome protective, tumor suppressor. These functions are lost or altered in a majority of malignancies. Loss of p53 predisposes individuals to familial, as well as acquired cancers in humans [51–53]. Nearly 1/3 of BCs harbor genetic lesions in p53 (Fig. 1) [54], and patients with BRCA mutations have a high likelihood of p53 mutations [55,56]. Patients who develop metastatic disease after primary therapy for earlier stages of BC have a high frequency of p53 mutations. The tumor suppressor functions of p53 are manifest in mice by a 100% incidence of lethal malignancy at < 24 weeks of age in homozygous p53 knockout ($p53^{-/-}$) mice. $RLIP^{-/-}$ and $p53^{-/-}$ mice are polar opposites in the spectrum of cancer susceptibility. We have discovered that RLIP knockout mice are resistant to cancer and obesity [21,33,57–60], and that depletion of RLIP by antisense in p53 knockout mice completely prevents the normal inevitable formation of lymphoma in these animals [57]. Until then, no prior genetic or drug intervention had been able to completely eliminate spontaneous cancers and normalize the gene expression profile in $p53^{-/-}$ mice [57]. The implication of these findings is that RLIP is necessary for translating carcinogenic or obesogenic stimuli into the respective clinical disease processes. Thus, RLIP could serve as a single target for the treatment of both cancer and diabetes.

4. Increased expression of RLIP in cancer cells and tissues

Clinical studies have demonstrated that RLIP mRNA and protein are highly overexpressed in BC tissues compared to adjacent normal mammary tissues and that RLIP protein expression is positively associated with the malignant stage of BC patients [35]. Wang *et al* showed that RLIP mRNA and protein expression are also positively correlated with glioma grade and that higher RLIP expression correlates with shorter patient survival [36]. The role of RLIP in cell cycle progression has also been reported. Yao *et al* found that reducing RLIP expression in leukemia cells using shRNA resulted in cell cycle arrest at G1 phase [39]. The anti-apoptotic effect of RLIP has been found in a number of human cancer cell lines, such as colon, glioma, and leukemia cell lines [38–40]. In addition, Wang *et al* proposed that RLIP may suppress apoptosis and promote the proliferation of glioma cells through direct ATP-dependent xenobiotic transport and by activating the Rac1-JNK signaling pathway [36]. On the other hand, suppressed cell invasion has been reported in colon cancer and glioma cells with RLIP knockdown (38, 40). RLIP depletion has also been found to inhibit tumor neovascularization in a mouse model of melanoma [33]. Lee *et al* suggested a role of RLIP in tumor cell induction of angiogenesis by demonstrating that RLIP regulates tumor cell transactivation of endothelial cells *via* control of VGEF expression and secretion [41]. Lee *et al* also suggested that the interaction between ARNO (a guanine nucleotide exchange factor for Arf6) and the RLIP N-terminus regulates cell spreading and motility *via* PI3K and Arf6 independently of RLIP control of Rac [42]. Moreover, *in vivo* studies using knockout mouse model showed that $RLIP^{-/-}$ mice were highly resistant to chemical carcinogenesis [21] and even resistant to the growth of subcutaneously implanted cancer cells [33]. Taken together, these studies indicate that high RLIP expression was associated with poor prognosis of cancer patients and suggest that RLIP act as an effector to promote tumor progression and metastasis.

5. RLIP antisense

RLIP is a fundamental link between biochemical pathways of GSH-linked metabolism of xenobiotics and stress-defense signaling pathways. It represents a stress-resistance effector that plays a pivotal role in defending normal cells from poisons, and cancer cells from apoptosis. Targeted inhibition or depletion of RLIP causes regression of human xenografts without inducing toxicity in nude mouse models. In this regard, we have developed biologically effective antisense technology to effectively cause inhibition of RLIP in breast tumor models. Antisense phosphorothioate technology is relatively straightforward and potentially high throughput method for inhibiting gene expression. The sequence to a specific mRNA can inhibit its expression by blocking the transfer of genetic information from DNA to protein. Antisense is a mature drug discovery and therapeutic platform, used *in vitro* and *in vivo* because of its stability in cells and tissues. Antisense drugs are designed to bind to the mRNA of a target protein and interfere with its associated mRNA, inhibiting the protein production process [61,62] (Fig. 2). We have demonstrated that RLIP antisense, which specifically down-regulates RLIP, a key stress-defense protein vital for survival of several histological types of cancer, is safe for administration, causes a transient increase in glucose uptake in cancer cells, and provide anticancer benefit through cancer cell-specific apoptosis. RLIP is a crucial anti-apoptotic protein that is particularly important for certain cancer cells to resist apoptosis [9–11]. Pharmacological implications include inhibiting the renal excretion of drugs, increasing accumulation of certain anti-epileptics in the brain, regulating insulin and cortisol signaling, and boosting resistance to radiant, oxidant, sheer, and electrical stress. Antisense therapy therefore is well positioned to play a significant role in drug development for human diseases [61,62]. These studies will also enable the development of an encompassing model of the mechanisms that control receptor-ligand signaling and lead to human clinical trials of RLIP-targeted therapy in BC. Since the pharmacokinetic properties of several phosphorothioate antisense molecules has been studied previously and shows short plasma half-lives but long-lasting tissue effects, and relatively predictable toxicities with respect to plasma drug-levels [61,62], we propose to target RLIP for depletion using RLIP antisense. We anticipate that normal tissue will not have the apoptotic effect seen in cancer cells after RLIP depletion, which seem to depend on RLIP for survival.

6. Targeting RLIP to overcome apoptosis resistance in BC

Despite several decades of effort and striking improvements to drug therapies targeting kinase signaling pathways, no effective and life-prolonging regimen of cytotoxic chemotherapy has been demonstrated for BC. BC remains a deadly malignancy if not found and removed in early stages. The failure of therapy frequently occurs because BC cells

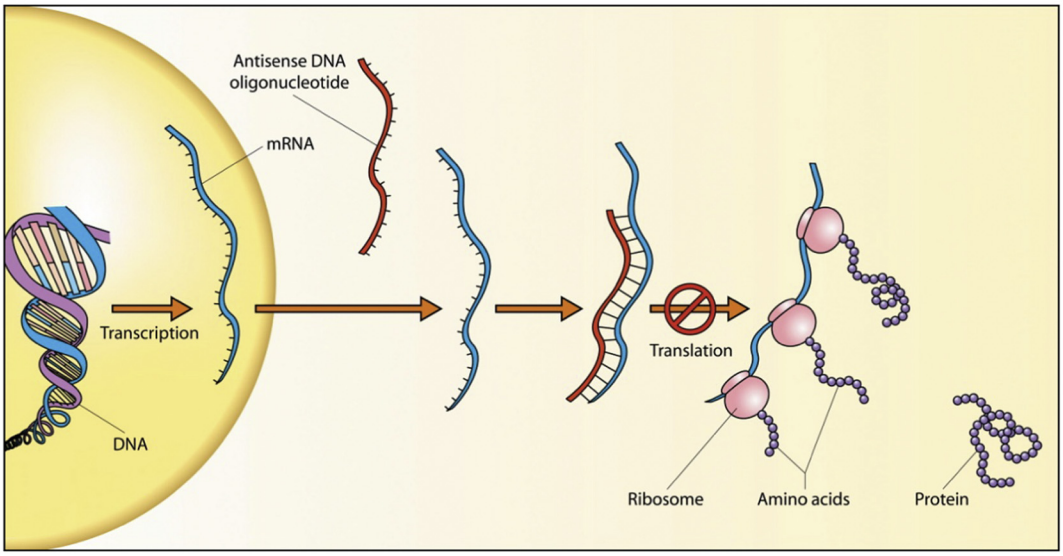

Fig. 2. RLIP antisense prevents mRNA translation: The antisense molecule binds with mRNA within the cell at nucleotides 508–528 preventing the full translation of the mRNA into normal RLIP protein. Without these nucleotides the protein cannot bind to the cell membrane causing the cessation of RLIP transport activity, leading cell death through lysis or apoptosis.

develop a phenotype of extreme resistance to the apoptotic effects of chemotherapy agents and targeted drugs, leading to frequent failure of therapy. This apoptosis-resistant phenotype is associated with myriad and complex changes in drug-transporter proteins, cell cycle checkpoint controls, DNA-repair, apoptosis signaling, and execution proteins. Attempts to increase the therapeutic efficacy of chemotherapy and targeted agents by modulating these known defense mechanisms has resulted in very limited success due to their inter-related and redundant nature.

Transporter proteins that are highly expressed in the membranes of BC cells can contribute to drug resistance; however, targeting the ABC transporter family protein has not been effective for reversing drug resistance in BC. The incidence of BC seems to be increasing, and early diagnosis and surgical therapy are the only effective cures. The highly drug- and radiation-resistant nature of BC, as compared to other neoplasms such as lung or colon cancer, is a major reason why there is still no effective and life-prolonging traditional cytotoxic chemotherapy for BC.

Cell line, animal, and human clinical data clearly indicate that the ABC-transporters BCRP, Pgp, MRP and related transporters are able to mediate drug-accumulation defects in malignant cells. BC is comprised of different entities, each being associated with distinct outcome and therapeutic approaches. However, correlations with pathology, clinical resistance, and outcomes in BC are poor, and attempts to improve therapeutic efficacy by targeting these have not been successful. This review article will provide a missing piece of the puzzle to the understanding of multi-specific transport mechanisms in BC, RLIP, a stress-responsive non-ABC, high capacity transporter, which must have had significant confounding effect in studies of ABC-transporters. Cancer cells appear significantly more sensitive to apoptosis triggered by blocking RLIP than normal cells, suggesting the feasibility of targeting RLIP in BC therapy. RLIP protein is greater in cancer cells as compared with normal cells, and that apoptosis triggered by RLIP inhibition will be manifested preferentially in cancer as compared with normal cells. The apoptosis-resistant phenotype is associated with increased signaling through cell survival and proliferation pathways (such as Akt and PKC) down-stream of extracellular ligands such as EGF, TGF, IGF1, and Wnt. These signaling pathways share a common regulatory mechanism: endocytosis of the receptor-ligand complex generally inhibits these pathways, but in some cases may be necessary for their activation (Fig. 3). Overall, RLIP represents a unique and potentially very useful target to globally disrupt survival signaling that underlies therapy resistance in BC. Thus, RLIP antisense could serve as a therapeutic agent by itself or in combination with other agents targeting cell-surface receptors.

7. Distribution of RLIP in cell membranes

RLIP is a stress-responsive GS-E transporter that is required for clathrin-dependent endocytosis (CDE). Using deletion mutants involving antennapedia homeodomain homologous sequences in the N-terminal of RLIP, we have identified an internal RLIP peptide sequence ($aa^{171–185}$) on the cell-surface in cancer cells [32]. Antibodies targeted at this epitope inhibit the transport activity of RLIP, causing apoptosis and intracellular accumulation of pro-apoptotic alkenals and xenobiotics [6,48,63]. Taken together, these studies show that RLIP is widely distributed in cell membranes and that the $aa^{171–185}$ epitope was found on the surface of BC cells.

8. Anti-neoplastic effects of targeting RLIP in breast cancer xenografts

The *in vitro* anticancer activity of RLIP antisense against BC cells suggests that RLIP expression in BC correlates with poor survival, contrary to predictions from the cBioportal analyses of the METABRIC study (Fig. 1). To confirm the results of the *in vitro* studies, we conducted *in vivo* studies to evaluate the anti-neoplastic efficacy of RLIP antisense, as well as RLIP antibodies, in xenograft mouse models bearing MCF7 and SKBR3 BC cells. Whereas tumors in control mice grew, tumors in mice treated with either RLIP antisense or anti-RLIP IgG shrank over the duration of the study. This reduction in size was confirmed by tumor weights after excision. Depletion of RLIP protein in the tumors of RLIP antisense-treated mice was confirmed by Western blot analysis of tumor homogenate using anti-RLIP IgG [15]. Immunohistochemical stains of tumor tissue sections showed remarkable disappearance of the proliferative marker Ki67 and the angiogenesis marker CD31. Expression of E-cadherin, a marker of differentiation that signifies reversal of epithelial-mesenchymal transition increased remarkably [15]. These results established the anticancer activity of targeting RLIP in BC. Blocking RLIP shuts down the toxic network of biochemical signals that promotes inflammation and cancer cell growth. Whereas cancer cells cannot survive without RLIP, normal cells

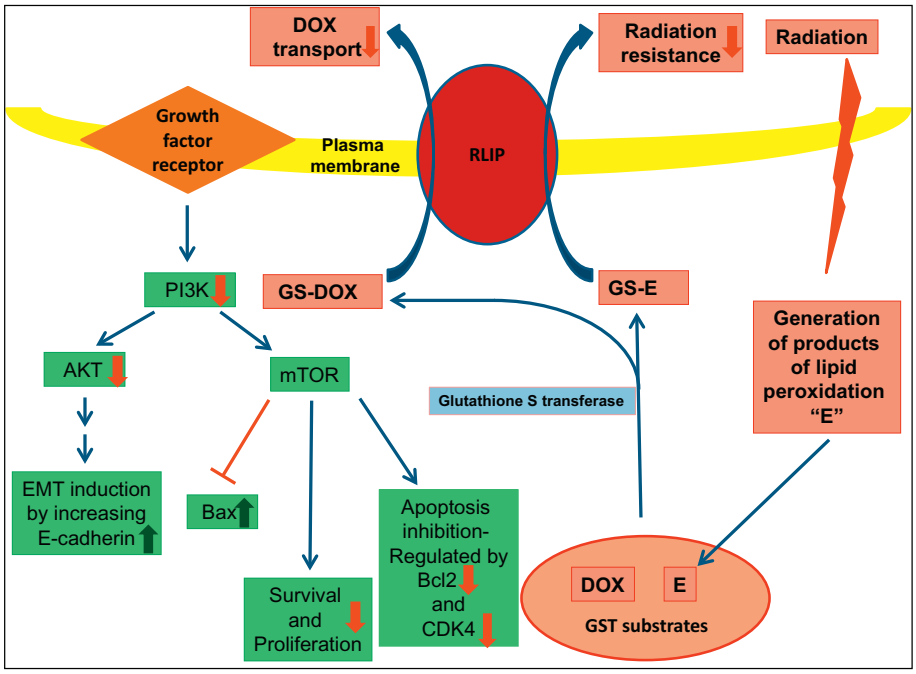

Fig. 3. Major mechanisms contributing to RLIP-mediated anticancer effects and chemo-radiotherapy resistance in breast cancer: RLIP depletion leads to predominant inhibition of the PI3K/Akt pathway, as revealed by the inhibition of PI3K, as well as a decrease in the level and phosphorylation of Akt. The associated down-stream proteins that regulate differentiation, proliferation, and apoptosis such as E-cadherin, Bax, Bcl2, and CDK4, are also differentially regulated by RLIP-targeting interventions. The collective impact of RLIP depletion on the regulation of cellular signaling pathways, as well as the detoxification of glutathione-electrophile conjugates (GS-Es) of lipid peroxidation and chemotherapy drugs, represents precious opportunity for the development of novel and effective therapeutic interventions for BC. *Green arrow*: up-regulation following RLIP depletion; *Red arrow*: down-regulation following RLIP depletion; *Blue arrow*: normal signal transduction.

do not need RLIP. RLIP, a stress-responsive, multi-functional protein with multi-specific transport activity, is frequently over-expressed in malignant cells. It plays a prominent anti-apoptotic role selectively in cancer cells. RLIP$^{-/-}$ mice are significantly more sensitive to radiation compared to RLIP$^{+/+}$ [22–24]. RLIP accounts for up to 80% of the transport activity, and blocking RLIP-mediated transport results in the accumulation of pro-apoptotic endogenous electrophiles and on-set of apoptosis [20,47]. These studies will lead to the recognition of RLIP as a major xenobiotic defense mechanism and advance the potential use of RLIP liposomes as pharmacological agents for use in humans to defend against the toxicity of xenobiotics and radiation.

9. Conclusion

BC is the second most common cancer worldwide after lung cancer, the fifth most common cause of cancer death, and the leading cause of cancer death in women. The global burden of BC exceeds all other cancers and the incidence rates of BC are increasing [1–3]. This fact reminds translational researchers of the challenging need to develop novel and effective therapies targeting molecules that regulate the proliferative, apoptotic, and drug sensitivity profiles of BC tumors. In this regard, one of the important factors that direct the development of interventional strategies for BCs is that, in spite of tremendous efforts by multiple research groups around the world, there are critical and unmet challenges in translating effective target molecules into biologically workable solutions that have immediate therapeutic relevance. In particular, breast tumors are characterized by a high degree of molecular heterogeneity, and aggressive subtypes like triple negative BC are known for a high degree of therapeutic refractoriness [64,65].

Lack of strict correlations between gene expression *vs.* protein expression (or catalytic function) and insufficient experimental or theoretical rationale limits the use of mRNA expression data from genomic analyses for identifying actionable targets that will have the greatest impact in cancer therapy. The theoretical rationale for targeting RLIP is based on several lines of reasoning and extensive experimental data from studies on the molecular and biological functions of RLIP by us and several other groups focused on RLIP [9–11,17–45]. These studies have established that RLIP is an allocrite-stimulated ATPase that is sufficient to mediate the transmembrane, anti-gradient, ATP hydrolysis-dependent transport of amphiphilic cations and anions across membranes [29,46,66–68]. The accumulation of intracellular GS-Es and other transported allocrites (anthracyclines, vinca alkaloids, and kinase-inhibitors) predicted by these studies has been verified [6,20,22–24,67]. Indeed, sensitivity to radiation, predicted based on the accumulation of lipid hydroperoxides and their metabolic product GS-Es, has been proven in knockout mouse models [22–24]. These studies provide the basis for a highly effective drug to prevent and treat radiation poisoning [69].

The studies outlined in the present review demonstrate the susceptibility of human BC cells to RLIP depletion or inhibition *in vitro* and *in vivo* [4,5,12–15]. In addition to its remarkably wide spectrum of activity, an existential role of RLIP in cancer is supported by the failure of RLIP knockout mice to develop cancer upon exposure to the powerful carcinogens benzo[*a*]pyrene and dimethylbenzanthracene (DMBA), which cause cancer in 100% of wild type mice [21]. Failure of syngeneic B16 melanoma cells to implant in RLIP knockout mice due to impaired angiogenesis indicate that carcinogenesis requires the presence of RLIP in cells of the stroma, as well as cancer parenchyma [33]. These studies using BC cells showed that RLIP deficiency inhibits endocytosis and down-stream signaling from EGF and Wnt to MAPK, ERK, and Myc, all of which are key proteins involved in the genesis and natural history of BC.

The diametrically opposite cancer susceptibility of RLIP$^{-/-}$ and p53$^{-/-}$ mice led us to explore a mutually inhibitory and functionally opposed relationship between RLIP and p53 in carcinogenesis. RLIP knockout homozygous (RLIP$^{-/-}$) mice have no spontaneous malignancy and are highly resistant to carcinogen-induced malignancy [21]. In stark contrast, p53 knockout homozygous (p53$^{-/-}$) mice have spontaneous malignancy in 100% by 6 months of age; similarly, p53 knockout heterozygous (p53$^{+/-}$) mice are also highly susceptible, with spontaneous malignancy by age 11–13 months of age and carcinogen-induced malignancy occurs at a much earlier age. Our recent studies revealed the astounding observation that p53$^{-/-}$ mice are entirely protected from spontaneous malignancy for up to 8 months of age by partial depletion of RLIP (an oncoprotein over-expressed in cancer cells) using antisense therapy. This protection was associated with nearly complete reversal of major epigenetic and related gene expression changes that occur normally in p53$^{-/-}$ mice [57]. Targeting RLIP to treat BC is a radically novel concept, the full implications of which are as yet unrealized. RLIP serves as a functional nexus between

glutathione-mediated defense mechanisms and signaling-mediated survival pathways [6,9,35–40].

10. Relevance to human health

Today, BC remains the leading cause of cancer deaths in females in the United States. RLIP represents a unique target in cancer therapy because its activation is the rate regulatory step in both MAP signaling and endocytosis. The former is crucial for protection of cells from stressors, including oxidation, chemical toxins, and radiation. The latter serves as a master regulator of receptor-ligand signaling. BC is characterized by the expression of multiple cell survival pathways that are down-stream of receptor-tyrosine kinase and Wnt signaling. RLIP depletion by antisense has been shown to be safe in animal studies [9–11,17,19,25,27], and to cause dramatic regression of BC [4,5,15]. Although indirect evidence and theoretical considerations indicate that RLIP likely serves as a master modulator of receptor-ligand signaling, it remains to be proven; BC is the ideal model for such studies. Understanding the roles RLIP plays in defense against stress (oxidative, chemical, and radiant) and in signaling (through its linkage with endocytosis) will enable the development of RLIP-targeting therapies as novel therapeutic agents capable of eradicating BC clinically. These are important studies as they would provide an opportunity to treat BC patients using a novel, targeted therapeutic approach that offers remission of tumors and a potential cure for the disease. These studies will establish a new generation of anticancer agent such as RLIP antisense phosphorothioate, against BC and can thus substantially enhance the quality of life and the survival of BC patients.

11. Future perspectives

The drug agents for targeting RLIP have not been fully developed. The ability of RLIP to transport a wide variety of anti-neoplastic agents renders it a multidrug transporter. Other multidrug transporters have not proven to be 'druggable,' primarily because of toxicity. This may not be an issue for RLIP, but remains to be determined. However, new generation antisense molecules could be considered. Though liposomal or other formulations were not required in mice, they may be required for humans. In summary, the development of RLIP-targeting therapies for BC appears justified based on the demonstrated sensitivity of BC, lack of significant toxicity in mice, and a reasonably well-defined mechanism of action that involves multiple key cancer-related signaling pathways. Based on these studies, the selection of patients on the basis of RLIP expression may not be required, but the presence of genetic alterations in related signaling molecules may be useful as predictive biomarkers to select patients who are likely to benefit from treatment.

12. Potential clinical impact

There are multiple subtypes of BC with distinct morphologies and clinical implications. The global burden of BC exceeds the burden impacted due to all other cancers [1–3]. The rate of BC continues to increase and with the interplay of enhancement of the environmental risk factors, dietary habits and contributing hormonal factors, the complex signaling cross-talk in many areas of pathogenesis, progression and therapeutic response in BCs still remain to be understood and targeted for developing effective clinical interventions. The incidence of early BC remains to be a major concern that needs extensive and meticulous care in view of the potential life events and the impact of progressive disease on attenuating quality and duration of further life span [70–72]. Hormone refractory subtypes of BC, such as triple negative BC, presents with significant challenges for effective clinical interventions. The absence of ER, PR, and HER2 in aggressive triple negative BC makes chemotherapy and radiotherapy the natural choice over hormone and HER2 antibody therapy [72–75]. Thus, we need to employ strategies to target the key molecules that regulate the incidence, progression, and drug- and radiation-resistance of BCs to develop an effective interventional agent for the complete spectrum of BCs. Such an agent should be well tolerated by normal cells without causing significant toxicity in tested animals. In this regard, RLIP-targeting interventions like RLIP antisense hold key promise for the treatment of BCs, including aggressive triple negative BCs. Therefore, effective antisense therapy is well positioned to play a significant role in development of drugs for human diseases. The body of work reviewed here demonstrates that RLIP is a very promising target for BC therapy.

Conflict of interest

No conflict of interest exists for any of the authors.

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, CA Cancer J. Clin. 66 (2016) 7–30.
[2] A.M. Noone, N. Howlader, M. Krapcho, D. Miller, A. Brest, M. Yu, J. Ruhl, Z. Tatalovich, A. Mariotto, D.R. Lewis, H.S. Chen, E.J. Feuer, K.A. Cronin (eds). SEER Cancer Statistics Review, 1975-2015, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2015/, (based on April 16, 2018 SEER data submission, posted to the SEER web site, April 2018).
[3] W. Chen, A.D. Hoffmann, H. Liu, X. Liu, Organotropism: new insights into molecular mechanisms of breast cancer metastasis, Precis. Oncol. 2 (2018) 4.
[4] J. Singhal, P. Singhal, D. Horne, R. Salgia, S. Awasthi, S.S. Singhal, Metastasis of breast tumor cells to brain is suppressed by targeting RLIP alone and in combination with 2′-hydroxyflavanone, Cancer Lett. 438 (2018) 144–153.
[5] J. Singhal, S. Chikara, D. Horne, R. Salgia, S. Awasthi, S.S. Singhal, RLIP inhibition suppresses breast-to-lung metastasis, Cancer Lett. 447 (2019) 24–32.
[6] S. Awasthi, S.S. Singhal, R. Sharma, P. Zimniak, Y.C. Awasthi, Transport of glutathione conjugates and chemotherapeutic drugs by RLIP76 (RALBP1): a novel link between G-protein and tyrosine kinase signaling and drug resistance, Int. J. Cancer 106 (2003) 635–646.
[7] Y.C. Awasthi, R. Sharma, S. Yadav, S. Dwivedi, A. Sharma, S. Awasthi, The non-ABC drug transporter RLIP76 (RALBP-1) plays a major role in the mechanisms of drug resistance, Curr. Drug Metab. 8 (2007) 315–323.
[8] S. Yadav, E. Zajac, S.S. Singhal, S. Awasthi, Linking stress-signaling, glutathione metabolism, signaling pathways and xenobiotic transporters, Cancer Metastasis Rev. 26 (2007) 59–69.
[9] S. Awasthi, S.S. Singhal, Y.C. Awasthi, B. Martin, J.-H. Woo, C.C. Cunningham, A.E. Frankel, RLIP76 and Cancer, Clin. Cancer Res. 14 (2008) 4372–4377.
[10] S.S. Singhal, S. Yadav, C. Roth, J. Singhal, RLIP76: a novel glutathione-conjugate and multi-drug transporter, Biochem. Pharmacol. 77 (2009) 761–769.
[11] S.S. Singhal, Y.C. Awasth, S. Awasthi, Regression of melanoma in a murine model by RLIP76 depletion, Cancer Res. 66 (2006) 2354–2360.
[12] S.S. Singhal, J. Singhal, M.P. Nair, A.G. Lacko, Y.C. Awasthi, S. Awasthi, Doxorubicin transport by RALBP1 and ABCG2 in lung and breast cancer, Int. J. Oncol. 30 (2007) 717–725.
[13] J. Singhal, L. Nagaprashantha, S. Chikara, S. Awasthi, D. Horne, S.S. Singhal, 2'-Hydroxyflavanone: a novel strategy for targeting breast cancer, Oncotarget 8 (2017) 75025–75037.
[14] L. Nagaprashantha, J. Singhal, H. Li, C. Warden, X. Liu, D. Horne, S. Awasthi, R. Salgia, S.S. Singhal, 2'-Hydroxyflavanone effectively targets RLIP76-mediated drug transport and regulates critical signaling networks in breast cancer, Oncotarget 9 (2018) 18053–18068.
[15] J. Singhal, S. Chikara, D. Horne, R. Salgia, S. Awasthi, S.S. Singhal, 2'-Hydroxyflavanone inhibits *in vitro* and *in vivo* growth of breast cancer cells by targeting RLIP76, Mol. Carcinogen. 57 (2018) 1751–1762.
[16] L. Nagaprashantha, R. Adhikari, J. Singhal, S. Chikara, S. Awasthi, D. Horne, S.S. Singhal, Translational advances and opportunities for broad-spectrum natural phytochemicals and targeted agent combinations in breast cancer, Int. J. Cancer 142 (2018) 658–670.
[17] S.S. Singhal, J. Singhal, S. Yadav, S. Dwivedi, P.J. Boor, Y.C. Awasthi, S. Awasthi, Regression of lung and colon cancer xenografts by depleting or inhibiting RLIP76 (Ral-binding protein 1), Cancer Res. 67 (2007) 4382–4389.
[18] S.S. Singhal, S. Yadav, J. Singhal, E. Zajac, Y.C. Awasthi, S. Awasthi, Depletion of RLIP76 sensitizes lung cancer cells to doxorubicin, Biochem. Pharmacol. 70 (2005) 481–488.

[19] S.S. Singhal, C. Roth, K. Leake, J. Singhal, S. Yadav, S. Awasthi, Regression of prostate cancer xenografts by RLIP76 depletion, Biochem. Pharmacol. 77 (2009) 1074–1083.
[20] D. Stuckler, J. Singhal, S.S. Singhal, S. Yadav, Y.C. Awasthi, S. Awasthi, RLIP76 transports vinorelbine and mediates drug resistance in non-small cell lung cancer, Cancer Res. 65 (2005) 991–998.
[21] S.S. Singhal, D. Wickramarachchi, S. Yadav, J. Singhal, K. Leake, R. Vatsyayan, P. Lelsani, P. Chaudhary, S. Suzuki, S. Yang, Y.C. Awasthi, S. Awasthi, Glutathione-conjugate transport by RLIP76 is required for clathrin-dependent endocytosis and chemical carcinogenesis, Mol. Cancer Ther. 10 (2011) 16–28.
[22] S. Awasthi, S.S. Singhal, S. Yadav, J. Singhal, K. Drake, A. Nadkar, E. Zajac, D. Wickramarachchi, N. Rowe, A. Yacoub, P. Boor, S. Dwivedi, P. Dent, W. Jarman, B. John, Y.C. Awasthi, RLIP76 is a major determinant of radiation sensitivity, Cancer Res. 65 (2005) 6022–6028.
[23] S.S. Singhal, S. Yadav, J. Singhal, M. Sahu, A. Sehrawat, S. Awasthi, Diminished drug transport and augmented radiation sensitivity caused by loss of RLIP76, FEBS Lett. 582 (2008) 3408–3414.
[24] J. Singhal, S.S. Singhal, S. Yadav, M. Warnke, A. Yacoub, P. Dent, R. Sharma, Y.C. Awasthi, RLIP76 in defense of radiation poisoning, Int. J. Rad. Oncol. Biol. Phys. 72 (2008) 553–561.
[25] K. Leake, J. Singhal, L.D. Nagaprashantha, S. Awasthi, S.S. Singhal, RLIP76 regulates PI3K/Akt signaling and chemo-radiotherapy resistance in pancreatic cancer, PLoS One 7 (2012) e34582.
[26] J. Singhal, S. Yadav, L. Nagaprashantha, R. Vatsyayan, S.S. Singhal, S. Awasthi, Targeting p53 null neuroblastomas through RLIP76, Cancer Prev. Res. 4 (2011) 879–889.
[27] S.S. Singhal, J. Singhal, S. Yadav, M. Sahu, Y.C. Awasthi, S. Awasthi, RLIP76: a target for kidney cancer therapy, Cancer Res. 69 (2009) 4244–4251.
[28] S.S. Singhal, A. Sehrawat, M. Sahu, P. Singhal, R. Vatsyayan, P.C. Rao Lelsani, S. Yadav, S. Awasthi, Rlip76 transports sunitinib and sorafenib and mediates drug resistance in kidney cancer, Int. J. Cancer 126 (2010) 1327–1338.
[29] S. Awasthi, J.-Z. Cheng, S.S. Singhal, U. Pandya, R. Sharma, S.V. Singh, P. Zimniak, Y.C. Awasthi, Functional reassembly of ATP-dependent xenobiotic transport by the N- and C-terminal domains of RLIP76 and identification of ATP binding sequences, Biochemistry 40 (2001) 4159–4168.
[30] S.S. Singhal, S. Yadav, K. Drake, J. Singhal, S. Awasthi, Hsf-1 and POB1 induce drug sensitivity and apoptosis by inhibiting, J. Biol. Chem. 283 (2008) 19714–19729.
[31] S.S. Singhal, S. Yadav, R. Vatsyayan, P. Chaudhary, J. Borva, J. Singhal, S. Awasthi, Increased expression of cdc2 inhibits transport function of RLIP76 and promotes apoptosis, Cancer Lett. 283 (2009) 152–158.
[32] S. Yadav, S.S. Singhal, J. Singhal, D. Wickramarachchi, E. Knutson, T.B. Albrecht, Y.C. Awasthi, S. Awasthi, Identification of membrane-anchoring domains of RLIP76 using deletion mutant analyses, Biochemistry 43 (2004) 16243–16253.
[33] S. Lee, J.G. Wurtzel, S.S. Singhal, S. Awasthi, L.E. Goldfinger, RALBP1/RLIP76 depletion in mice suppresses tumor growth by inhibiting tumor neovascularization, Cancer Res. 72 (2012) 5165–5173.
[34] Y. Hu, N.F. Mivechi, HSF-1 interacts with Ral-binding protein 1 in a stress-responsive, multiprotein complex with HSP90 *in vivo*, J. Biol. Chem. 278 (2003) 17299–17306.
[35] C.Z. Wang, P. Yuan, B. Xu, L. Yuan, H.-Z. Yang, X. Liu, RLIP76 expression as a prognostic marker of breast cancer, Eur. Rev. Med. Pharmacol. Sci. 19 (2015) 2105–2111.
[36] Q. Wang, J.Y. Wang, X.P. Zhang, Z.W. Lv, D. Fu, Y.C. Lu, RLIP76 is overexpressed in human glioblastomas and is required for proliferation, tumorigenesis and suppression of apoptosis, Carcinogenesis 34 (2013) 916–926.
[37] W. Zhong, C. Owens, N. Chandra, K. Popovic, M. Conaway, D. Theodorescu, RalBP1 is necessary for metastasis of human cancer cell lines, Neoplasia 12 (2010) 1003–1012.
[38] Q. Wang, J. Qian, J. Wang, C. Luo, J. Chen, G. Hu, Y. Lu, Knockdown of RLIP76 expression by RNA interference inhibits invasion, induces cell cycle arrest, and increases chemosensitivity to the anticancer drug temozolomide in glioma cells, J. Neuro-Oncol. 112 (2013) 73–82.
[39] K. Yao, H. Xing, W. Yang, A. Liao, B. Wu, Y. Li, R. Zhang, Z. Liu, Knockdown of RLIP76 expression by RNA interference inhibits proliferation, enhances apoptosis, and increases chemosensitivity to daunorubicin in U937 leukemia cells, Tumour Biol. 35 (2014) 8023–8031.
[40] Y. Zhang, X. Song, W. Gong, Z. Zhu, X. Liu, Q. Hou, Y. Sun, J. Chai, L. Zou, J. Guan, RLIP76 blockade by siRNA inhibits proliferation, enhances apoptosis, and suppresses invasion in HT29 colon cancer cells, Cell Biochem. Biophys. 71 (2015) 579–585.
[41] S. Lee, L.E. Goldfinger, RLIP76 regulates HIF-1 activity, VGEF expression and secretion in tumor cells, and secretome transactivation of endothelial cells, FASEB J. 28 (2014) 4158–4168.
[42] S. Lee, J.G. Wurtzel, L.E. Goldfinger, The RLIP76 N-terminus binds ARNO to regulate PI3K, Arf6 and Rac signaling, cell spreading and migration, Biochem. Biophys. Res. Commun. 454 (2014) 560–565.
[43] S.B. Cantor, T. Urano, L.A. Feig, Identification and characterization of Ral-binding protein 1, a potential downstream target of Ral GTPases, Mol. Cell. Biol. 15 (1995) 4578–4584.
[44] V. Jullien-Flores, O. Dorseuil, F. Romero, F. Letourneur, S. Saragosti, R. Berger, A. Tavitian, G. Gacon, J.H. Camonis, Bridging Ral GTPase to Rho pathways. RLIP76, a Ral effector with CDC42/Rac GTPase-activating protein activity, J. Biol. Chem. 270 (1995) 22473–22477.
[45] J.G. Wurtzel, S. Lee, S.S. Singhal, S. Awasthi, M.H. Ginsberg, L.E. Goldfinger, RLIP76 regulates Arf6-dependent cell spreading and migration by linking ARNO with activated R-Ras at recycling endosomes, Biochem. Biophys. Res. Commun. 467 (2015) 785–791.
[46] S. Awasthi, J. Cheng, S.S. Singhal, M.K. Saini, U. Pandya, S. Pikula, J. Pikula, S.V. Singh, P. Zimniak, Y.C. Awasthi, Novel function of human RLIP76: ATP-dependent transport of glutathione-conjugates and doxorubicin, Biochemistry 39 (2000) 9327–9334.
[47] S. Awasthi, S.S. Singhal, J. Singhal, J. Cheng, P. Zimniak, Y.C. Awasthi, Role of RLIP76 in lung cancer doxorubicin resistance: doxorubicin transport in lung cancer by RLIP76, Int. J. Oncol. 22 (2003) 713–720.
[48] S. Awasthi, S.S. Singhal, J. Singhal, Y. Yang, P. Zimniak, Y.C. Awasthi, Role of RLIP76 in lung cancer doxorubicin resistance: anti-RLIP76 antibodies trigger apoptosis in lung cancer cells and synergistically increase doxorubicin cytotoxicity, Int. J. Oncol. 22 (2003) 721–732.
[49] J. Singhal, L.D. Nagaprashantha, R. Vatsyayan, S. Awasthi, S.S. Singhal, RLIP76, a glutathione-conjugate transporter, plays a major role in the pathogenesis of metabolic syndrome, PLoS One 6 (2011) e24688.
[50] B. Maiti, N.M. Kundranda, T.P. Spiro, H.A. Daw, The association of metabolic syndrome with triple-negative breast cancer, Breast Cancer Res. Treat. 121 (2010) 479–483.
[51] P.L. Chen, Y.M. Chen, R. Bookstein, W.H. Lee, Genetic mechanisms of tumor suppression by the human p53 gene, Science 250 (1990) 1576–1580.
[52] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.
[53] K.T. Bieging, S.S. Mello, L.D. Attardi, Unravelling mechanisms of p53-mediated tumor suppression, Nat. Rev. Cancer 14 (2014) 359–370.
[54] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, Y. Sun, A. Jacobsen, R. Sinha, E. Larsson, E. Cerami, C. Sande, N. Schultz, Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) l1.
[55] F. Fons Elstrodt, A. Hollestelle, J.H.A. Nagel, BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants, Cancer Res. 66 (2006) 41–45.
[56] L.J. van 't Veer, H. Dai, M.J. van de Vijver, Y.D. He, A.A. Hart, M. Mao, H.L. Peterse, K. van der Kooy, M.J. Marton, A.T. Witteveen, G.J. Schreiber, R.M. Kerkhoven, C. Roberts, P.S. Linsley, R. Bernards, S.H. Friend, Gene expression profiling predicts clinical outcome of breast cancer, Nature 415 (2002) 530–536.
[57] S. Awasthi, J. Tompkins, J. Singhal, A.D. Riggs, S. Yadav, X. Wu, S. Singh, C. Warden, Z. Liu, J. Wang, T.P. Slavin, J.N. Weitzel, Y.-C. Yuan, M. Awasthi, S.K. Srivastava, Y.C. Awasthi, S.S. Singhal, Rlip depletion prevents spontaneous neoplasia in TP53 null mice, Proc. Natl. Acad. Sci. 115 (2018) 3918–3923.
[58] S. Awasthi, S.S. Singhal, S. Yadav, J. Singhal, R. Vatsyayan, E. Zajac, R. Luchowski, J. Borvak, K. Gryczynski, Y.C. Awasthi, A central role of RLIP76 in regulation of glycemic control, Diabetes 59 (2010) 714–725.
[59] S. Chikara, L. Nagaprashantha, J. Singhal, D. Horne, S. Awasthi, S.S. Singhal, Oxidative stress and dietary phytochemicals: role in cancer chemoprevention and treatment, Cancer Lett. 413 (2018) 122–134.
[60] S.S. Singhal, J. Figarola, J. Singhal, M.A. Reddy, X. Liu, D. Berz, R. Natarajan, S. Awasthi, RLIP76 protein knockdown attenuates obesity due to a high-fat diet, J. Biol. Chem. 288 (2013) 23394–23406.
[61] E. Uhlmann, A. Peyman, Antisense oligonucleotides: a new therapeutic principle, Chem. Rev. 90 (1990) 543–584.
[62] C.F. Bennett, E.E. Swayze, RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 259–293.
[63] J.Z. Cheng, R. Sharma, Y. Yang, S.S. Singhal, A. Sharma, M.K. Saini, S.V. Singh, P. Zimniak, S. Awasthi, Y.C. Awasthi, Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress, J. Biol. Chem. 276 (2001) 41213–41223.
[64] J.D. Brenton, L.A. Carey, A.A. Ahmed, C. Caldas, Molecular classification and molecular forecasting of breast cancer: ready for clinical application? J. Clin. Oncol. 23 (2005) 7350–7360.
[65] J.S. Reis-Filho, A.N.J. Tutt, Triple negative tumours: a critical review, Histopathology 52 (2008) 108–118.
[66] S. Awasthi, S.S. Singhal, S.K. Srivastava, R.T. Torman, P. Zimniak, J. Bandorowicz-Pikula, S.V. Singh, J.T. Piper, Y.C. Awasthi, S. Pikula, ATP-dependent human erythrocyte glutathione-conjugate transporter. Purification, photoaffinity labeling, and kinetic characteristics of ATPase activity, Biochemistry 37 (1998) 5231–5238.
[67] S. Awasthi, S.S. Singhal, S.K. Srivastava, P. Zimniak, K.K. Bajpai, M. Saxena, R. Sharma, S.A. Ziller, E.P. Frenkel, S.V. Singh, N.G. He, Y.C. Awasthi, Adenosine triphosphate-dependent transport of doxorubicin, daunomycin, and vinblastine in human tissues by a mechanism distinct from the P-glycoprotein, J. Clin. Invest. 93 (1994) 958–965.
[68] S. Awasthi, S.S. Singhal, S. Pikula, J.T. Piper, S.K. Srivastava, R.T. Torman, J. Bandorowicz-Pikula, J.T. Lin, S.V. Singh, P. Zimniak, Y.C. Awasthi, ATP-dependent human erythrocyte glutathione-conjugate transporter. Functional reconstitution of transport activity, Biochemistry 37 (1998) 5239–5248.
[69] P.G. Prasanna, D. Narayanan, K. Hallett, E.J. Bernhard, M.M. Ahmed, G. Evans, B. Vikram, M. Weingarten, C.N. Coleman, Radio-protectors and radio-mitigators for improving radiation therapy: the Small Business Innovation Research (SBIR) gateway for accelerating clinical translation, Radiat. Res. 184 (2015) 235–248.
[70] J.R. Benson, I. Jatoi, M. Keisch, F.J. Esteva, A. Makris, V.C. Jordan, Early breast cancer, Lancet 373 (2009) 1463–1479.
[71] G.V. Dall, K.L. Britt, Estrogen effects on the mammary gland in early and late life and breast cancer risk, Fron. Oncol. 7 (2017) 110.
[72] X. Dai, T. Li, Z. Bai, Y. Yang, X. Liu, J. Zhan, B. Shi, Breast cancer intrinsic subtype classification, clinical use and future trends, Am. J. Cancer Res. 5 (2015)

2929–2943.
[73] L. Hutchinson, Breast cancer: challenges, controversies, breakthroughs, Nat. Rev. Clin. Oncol. 7 (2010) 669–670.
[74] M. Colleoni, B.F. Cole, G. Viale, M.M. Regan, K.N. Price, E. Maiorano, Classical cyclophosphamide, methotrexate, and fluorouracil chemotherapy is more effective in triple-negative, node negative breast cancer: results from two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer, J. Clin. Oncol. 28 (2010) 2966–2973.
[75] D.C. Allred, Issues and updates: evaluating estrogen receptor-alpha, progesterone receptor, and HER2 in breast cancer, Mod. Pathol. 23 (2010) S52–S59.