Interdisciplinary Cancer Research 7

Nima Rezaei *Editor*

Breast Cancer Treatment: An Interdisciplinary Approach

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Volume 7

The “Interdisciplinary Cancer Research” series publishes comprehensive volumes on different cancers and presents the most updated and peer-reviewed articles on human cancers. Over the past decade, increased cancer research has greatly improved our understanding of the nature of cancerous cells which has led to the development of more effective therapeutic strategies to treat cancers. This translational series is of special value to researchers and practitioners working on cell biology, immunology, hematology, biochemistry, genetics, oncology and related fields.

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Editor

Breast Cancer Treatment: An Interdisciplinary Approach

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Preface

There are different types of treatment for breast cancer, including surgery, chemotherapy, radiotherapy, hormone therapy, immunotherapy, and targeted therapy. Deciding on the types of treatment depends on several factors, such as the stage and grade of cancer, biomarkers, and general health of patients.

The "Interdisciplinary Cancer Research" series publishes comprehensive volumes on different cancers. It plans to present the most updated and peer-reviewed interdisciplinary chapters on cancers. This interdisciplinary book series is of special value to researchers and practitioners working on cell biology, immunology, hematology, biochemistry, genetics, oncology and related fields. This is the main concept of Cancer Immunology Project (CIP) and Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), which are two active interest groups of the Universal Scientific Education and Research Network (USERN).

The seventh volume of the book entitled *Breast Cancer Treatment: An Interdisciplinary Approach* starts with a general chapter on treatment of triple-negative breast cancer (TNBC) and ductal carcinoma in situ (DCIS), followed by chapters on ablative breast cancer surgery, radiotherapy, and percutaneous breast cancer treatment. Revolutionizing breast cancer care is the subject of Chapter 6. Hormone therapy and hormone immunotherapy combination are discussed in Chapters 7–10. The other Chapters (11–17) present potential targeted therapies for breast cancer. Finally, the last chapter presents a case study on holistic nursing care for a postoperative breast cancer patient with lymphedema.

I hope that this interdisciplinary book will be comprehensible, cogent, and of special value for researchers and oncologists who wish to extend their knowledge on breast cancer treatment.

Tehran, Iran — Nima Rezaei

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About the Editor

Nima Rezaei MD, PhD, gained his medical degree (MD) from Tehran University of Medical Sciences and subsequently obtained an MSc in Molecular and Genetic Medicine and a PhD in Clinical Immunology and Human Genetics from the University of Sheffield, UK. He also spent a short-term fellowship of Pediatric Clinical Immunology and Bone Marrow Transplantation in the Newcastle General Hospital. Professor Rezaei is now the Full Professor of Immunology and Vice Dean of Research and Technologies, School of Medicine, Tehran University of Medical Sciences, and the co-founder and Head of the Research Center for Immunodeficiencies. He is also the Founder of Universal Scientific Education and Research Network (USERN). Professor Rezaei has already been the Director of more than two hundred research projects and has designed and participated in several international collaborative projects. Professor Rezaei is the editor, editorial assistant, or editorial board member of more than 50 international journals. He has edited more than one hundred international books, has presented more than a thousand lectures/posters in congresses/meetings, and has published more than 1500 scientific papers in international journals.

Signal Transducer and Activator of Transcription as a Potential Therapeutic Target in Breast Cancer

Niloofar Deravi and Nima Rezaei

Abstract

Signal transducer and activator of transcription (STAT) signaling pathway, connected upstream with Janus kinases (JAK) proteins, is known to be capable of the integration of inputs from various signaling pathways. Each family member exerts a particular role in signal transduction and is hence crucial to mediate different cellular responses to cytokines. STAT proteins especially STAT3 and STAT 5 are widely investigated and known to be involved in breast cancer progression. On the other hand, STAT1 and STAT4 exert opposite roles through suppression of breast tumor growth. Persistent activation of STAT3/5 can promote chronic inflammation, increasing healthy cells susceptibility to carcinogenesis. In this chapter, we discuss the structure, functional development, and activation of STAT family members. We also summarize the roles of different STAT proteins in breast cancer and its different subtypes. We also discuss STAT signaling pathway inhibitors used to treat breast cancer.

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1 Introduction

Breast cancer, ranked first in terms of new cases and deaths, is one of the leading cancers affecting women worldwide (Siegel et al. 2020). Due to the development in diagnostic tests for identifying ER expression models and amplification of human epidermal growth factor receptor 2 (HER2) in human breast cancer clinical samples, pathologists have classified breast cancer into three major subtypes: (1) HER2 amplified, (2) estrogen receptor (ER)-positive, and (3) triple-negative breast cancer (TNBC), which is ER-negative with the absence of amplification of HER2 amplification (Ross et al. 2009; Bertos and Park 2011; Guiu et al. 2012).

According to the profiling of gene expression, we can divide ER-positive subtype into luminal A and B subtypes; TNBC can also be subdivided into six other subtypes (Lehmann et al. 2011; Prat and Perou 2011; Eroles et al. 2012; Geyer et al. 2012; Koboldt et al. 2012). Additional subtyping of luminals and TNBC led to revealing various prognostic subtypes, some of which due to differences in both macro-and micro-environment and some due to intrinsic varieties in gene expression in cancer cells (Bertos and Park 2011). In TNBCs, poor prognosis has been linked with low expression of claudin in the cancer cells (Lu et al. 2013), while better prognosis is connected with higher presence of B-cell and low action in interleukin 8 (IL-8) (Rody et al. 2011). In order to establish more refined prognostic and therapeutic markers, molecular investigations have been conducted on luminal A and B ER-positive subtypes and HER2-amplified subtypes (Jönsson et al. 2010; Geyer et al. 2012).

In the early stages of breast cancer, some patients are treated via surgery; however, most of whom require postoperative chemotherapy, endocrine therapy, and radiotherapy in addition to surgery. These conventional treatments have for a long-time prolonged patient's survival and still hold a prominent position in the treatment of the disease, but they would also show inevitable adverse effects. Symptoms associated with chemotherapy, endocrine therapy, and radiotherapy make a patient's quality of life even worse. The two most common chemotherapeutic drugs used in breast cancer are anthracyclines and taxanes. Anthracyclines are the most general used and useful chemotherapy drugs for breast cancer. Anthracyclines have thus become an essential adjuvant and palliative medications. Anthracyclines can have a variety of toxic side effects, such as alopecia, mucositis, myelosuppression, and possible permanent cardiovascular effects (Cai et al. 2019).

Conversely, endocrine therapy can be an important treatment for hormone-positive breast cancer, the most general type of breast cancer. However, it can also cause adverse effects, including hot flashes, night sweats, cognitive changes, and fatigue (Mouridsen 2006).

Radiotherapy subsequent to breast-conserving surgery can increase patients' survival. It also helps patients diagnosed with lymph node-positive breast cancer in a mastectomy (McGale et al. 2014). Nonetheless, irradiation adverse events such as erythema, erosion, edema, and ulceration are some inevitable adverse effects of radiotherapy. Radiotherapy-induced tissue damage may take a long time to repair, and muscular rigidity in the area of the radiotherapy may result in persistent limb impairment (Ding et al. 2018). Breast cancer treatment with targeted therapy is a relatively recent concept. It exerts a remarkable therapeutic impact compared to the abovementioned traditional therapeutic approaches, yet it has fewer side effects and may lead to minimal damage in normal tissues. Transcription factors (TFs) have domains that can bind to the DNA of certain gene promoter regions. Many TFs exert role in the genesis and development of breast cancer. STAT family is one of the common outstanding TF families in breast cancer. STAT family consists of seven structurally related and highly conserved members, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 (Furtek et al. 2016). STAT family members are attractive molecular targets in breast cancer therapy.

2 STAT Family Members Characteristics, Activation, and Functional Development

Several structurally and functionally conserved areas are found in all STAT proteins. During STAT monomer activation, the Src-homology 2 (SH2) domain, in conjunction with the N-terminal domain (ND), facilitates homo- and heterodimerization. Most STAT inhibitors target highly conserved SH2 domain. Subsequent to undergoing serine phosphorylation, C-terminal transcriptional activation domain (TAD) obtains further transcriptional activators, boosting STAT's transcriptional effect. The DNA-binding domain (DBD) determines DNA association. STAT proteins can constitute isoforms by either posttranslational processing of proteolytic or alternative splicing of mRNA; β, γ, or δ isoforms are the short isoforms, whereas α isoforms are full-length isoforms (Lim and Cao 2006).

STAT proteins, in their latent state, are inactive, situated in the unstimulated target cells cytoplasm as unphosphorylated dimers or monomers (Verhoeven et al. 2020). A variety of cytokines, such as interleukins, growth factors, interferons (IFNs), and hormones binding to membrane receptors can activate these proteins (Bromberg 2001; Schindler 2002). Various different and occasionally overlapping ligands activate STAT1, STAT3, STAT5a, and STAT5b, but only a few cytokines activate STAT2, STAT4, and STAT6. STATs can be activated in a variety of modalities. STATs activity is primarily mediated via the Janus kinase (JAK-STAT) pathway. Extracellular signaling polypeptides communicate with transmembrane receptors in this cascade, resulting in a method for transcriptional control without the utilization of secondary messengers. There is an association between interferon, interleukin, and hormone receptors and a tyrosine kinase of the JAK family (known as tyrosine kinase 2 (Tyk2)) as well as a tyrosine residue on their cytoplasmic domain. Coupling of receptor and ligand causes the receptors to dimerize or oligomerize,

which activates the linked JAK proteins. JAKs subsequently can phosphorylate trans-phosphorylate receptor-associated tyrosine residues and also themselves, providing docking sites for the SH2 domains in the latent cytoplasmic STAT. Interactions of SH2 and pTyr are very selective, and identifying the specificity of the activation of STAT meditated by receptors is a crucial step. At the time of the coupling, on the receptor-associated STAT molecules, the JAKs would phosphorylate a specific Tyr residue (known as pY), leading to the SH2 domain of the pSTAT associated with one receptor and a reciprocal binding of the pY of the pSTAT associated with the other receptor, causing STAT hetero- or homodimerization. The dimer of pSTAT and pSTAT is subsequently liberated from the receptors to the nucleus quickly via using importins (Schindler 2002). The translocated dimer is now capable of binding certain DNA regulatory elements (DREs), and, when identified, the target gene's rate of transcription will vary substantially (Aaronson and Horvath 2002; Lim and Cao 2006; Konjević 2009). Even though phosphorylation causes STAT dimerization, there is solid evidence that, with just a minimum fraction as monomers and multimeric complexes known as statosomes, unphosphorylated STATs persist primarily as latent homo- or heterodimers (Stancato et al. 1996; Novak et al. 1998; Ndubuisi et al. 1999; Haan et al. 2000; Braunstein et al. 2003). These latent dimers, like their phosphorylated counterparts, can shuttle between the cytoplasm and nucleus (Liu et al. 2005; Reich 2013) and regulate critical cellular activities (Yang et al. 2007; Cheon and Stark 2009; Carbognin et al. 2016; Park et al. 2016). STATs lack tyrosine kinase activity and hence rely on other molecules. Alternative STATs activation is possible through growth factor receptors and non-receptor proteins' intrinsic tyrosine kinase activity. The epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), via JAK-STAT signaling pathway, control apoptosis, proliferation, and differentiation, regulating stem cell maintenance, inflammatory response, embryonic development, and hematopoiesis response (Silvennoinen et al. 1993; Brooks et al. 2014; Thomas et al. 2015). Other ways of activation have been suggested for STATs, including adaptor proteins moving JAKs closer to STATs, activation via G-protein coupled receptors (GPCRs), and non-receptor tyrosine kinases. Further cellular pathways might enhance the original STAT-activating stimulus thanks to the complicated mechanisms of regulation given by multiple forms of activation (Decker and Kovarik 2000).

It is now known that the STAT can bind to various DREs throughout the genome, controlling various protein-coding genes transcription. The JAK-STAT pathway's targets include mostly genes involved in development, immunology, and stress and metabolic responses (Tsurumi et al. 2017). STATs bind to proximal DREs as classic transcription factors (TFs), causing transcriptional activation, repression, or neutral binding. They also engage more distal binding sites, far from protein-encoding genes, through which they control enhancer activity (regions of DNA that augment the activity of a promoter in an orientation- and position-independent manner, coordinating cell-type-specific gene expression, e.g., TFs) and (ii) epigenetic status of associated genes or (i) straightly guide the noncoding loci such as miRNAs or (ii) directly instruct non (37). STAT-induced epigenetic alterations are seen in areas that control binding of STAT and regulation of transcription. This creates an

autoregulatory mechanism with functional consequences that are either suppressing (such as histone H3 trimethylation at Lys27) or potentiating (such as histone H3 trimethylation at Lys4) (Mandal et al. 2011).

Because of epigenetic changes that allow the access of the STATs to genes, the aimed repertoire for a certain STAT protein might differ from one cell type to another. STAT1, STAT3, STAT4, and STAT6 found in immune cells (Wei et al. 2010; Qiao et al. 2013; Qiao et al. 2016) and STAT5 found in mammary epithelium have all been shown to exhibit epigenetic alterations (Kang et al. 2014; Willi et al. 2016). STAT protein-generated transcriptional activity is affected by other parameters, including interaction with other TFs, selectivity of the gene, genome-wide competition (for access to the same DRE, members of the STAT family compete and mediate different outcomes), heterogenous signaling of STATs (distinct outcomes by the same molecule), and intensity or duration of the signaling of the STATs signaling (Villarino et al. 2017). The transcriptional result created via the JAK-STAT signaling pathway, and consequently a cell or tissue response, is mediated by a large array of intrinsic variables.

2.1 STAT Family Members Expression and Activity in Breast Cancer

STAT family members were discovered at the same time as three key subtypes of breast cancer (including HER2-amplified, TNBC, and ER-positive) and before further subtyping by profiling of the genes' expression. As a result, previous studies on the expression of STATs in breast cancer did not necessarily reveal any parallels or variations in patterns of expression across all breast cancer subtypes. Nonetheless, these studies provide fundamental information on the frequency of STAT family members' activity and expression in breast cancer. STAT family members' activity and expression are controlled at various levels, such as phosphorylation of tyrosine, modification of transcription, and posttranslational, and their effect on function of cells varies depending on their nuclear or cytoplasmic location (Clevenger 2004; Vafaizadeh et al. 2012; Furth 2014). STAT3 and STAT5a/b downregulation via various agents is correlated with decreased growth in cell lines of breast cancer modeling various breast cancer subtypes (Lim et al. 2012; Park et al. 2012), recommending that STATs could act as therapeutic aim for various subtypes of breast cancer.

2.2 STAT1 and Breast Cancer

Research on the mammary glands of mice showed that the expression of STAT1 is kept during lactation, pregnancy, and involution, though DNA binding and Y701's phosphorylation are only identified in early pregnancy, late involution, and virgin animals (Watson 2001). STAT1 regulation occurs in various stages of breast development (Watson 2001), yet the regular development of mammary glands occurs in

$STAT1^{-/-}$ mice. Contrary to normal untransformed breast cells, STAT1 is involved in the development of mammary cancer due to the phosphorylation of STAT1 Y701 is increased in human mammary tumors (Yu and Jove 2004) and is correlated with elevated survival regardless of the rest of the prognostic factors (Widschwendter et al. 2002). Moreover, elevated levels of mRNA in STAT1 were demonstrated to be part of a molecular signature that is correlated with greater metastatic outcome prediction of patients with triple-negative and hormone receptor-negative mammary cancers (Yau et al. 2010).

Recent independent researches in Hennighausen (Klover et al. 2010), Koromilas (Raven et al. 2011), Schreiber (Chan et al. 2012), and Sexl (Schneckenleithner et al. 2011) clarified the STAT1 role in the formation of mammary tumors. In spite of the implementation of various experimental ways, all these studies led to similar outcomes and supported the STAT1 role in tumor suppression in breast tumorigenesis. Hennighausen et al. reported the generation of mice bearing a STAT1 floxed (flx) allele (Klover et al. 2010). The $STAT1^{flx/flx}$ mouse was crossed to a mouse with ErbB2/neu oncogene expression (deemed NIC) while controlling the promoter of mouse mammary tumor virus (MMTV) (Klover et al. 2010). Originally, the MMTV-NIC mouse was for the expression of Cre recombinase while being controlled by an internal ribosome entry site (IRES) from the same dicistronic mRNA as the NIC oncogene (MMTV-NIC-IRES-Cre) (Ursini-Siegel et al. 2008). Mating of $STAT1^{flx/flx}$ mice with MMTV-NIC-IRES-Cre mice on background of FVB led to deletion of STAT1 in the same epithelium cell of breast with NIC expression (Klover et al. 2010). First, after 36 of birth, tumors could be detected in both groups, though the total latency of the disease was remarkably improved in mice with STAT1 deficiency (49.4 weeks) compared with STAT1-proficient animals (62.4 weeks). Since STAT1 was expressed in all cells in the microenvironment of tumor using this way, the STAT1 antitumor role was thus undeniably associated with the cell-intrinsic features in the epithelium of breast.

The Koromilas group used a tumorigenesis model in vivo. In this model, mice expressed ErbB2/Neu's active form controlled by the MMTV promoter (Siegel et al. 1999) and, on background of Balb/c, were crossed with $STAT1^{+/+}$and $STAT1^{-/-}$ mice (Durbin et al. 1996). Moreover, Neu NDL2–5-positive $STAT1^{-/-}$ female mice who had borne one litter of pups were observed to develop tumors nearly 6 weeks sooner in comparison to $STAT1^{+/+}$ counterparts (27 weeks vs. 33 weeks) (Raven et al. 2011). The reduced tumor formation latency was intensified in virgin females; $STAT1^{-/-}$ mice developed tumors at an average of 41.6 weeks while the $STAT1^{+/+}$ mice with tumors at an average of 49.1 weeks. Once development of tumor was initiated, no detectable difference was found in the number or size of tumors (Raven et al. 2011). Consequently, STAT1 acted as a suppressor for the formation of Neu NDL2–5 mammary gland tumor in mice (Raven et al. 2011). Elevated formation of tumor in $STAT1^{-/-}$ mice did not occur due to changes in morphology of the breast gland in the animals that were consistent with former findings showing no defects in mammary development in $STAT1^{-/-}$ mice (Watson 2001).

The Schreiber and Sexl groups investigated the spontaneous growth of breast tumors to assess the effect of STAT1 in vivo (Schneckenleithner et al. 2011; Chan

et al. 2012). A greater tumor incidence above 90% was reported by the Schreiber group in the parous animals, while a tumor incidence of 55% was established by the Sexl group. Sexl et al. showed no tumor in virgin females, though Schreiber et al. reported a 65% incidence of tumor in virgin animals. Moreover, minute differences were shown in receptor expression in the tumors of both researches; in the Schreiber et al.'s study, tumors showed an estrogen receptor-positive $(ER)^+$ phenotype nearly resembling human progesterone receptor $(PR)^+/ER^+$ tumors, while in Sexl et al.'s study, only 50% of the $STAT1^{-/-}$ tumors were ER^+. The reason for these differences is not yet understood, though the different genetic backgrounds may be involved, i.e., 129S6/SvEV in the Schreiber et al.'s researches vs. Balb/c in the Sexl et al.'s researches.

Both researches of the Sexl and Schreiber labs revealed an improved manifestation of mammary intraepithelial neoplasias (MINs) from lack of STAT1 (Schneckenleithner et al. 2011; Chan et al. 2012). MINs denote precancerous lesions that may potentially develop carcinomas and are considered to be a consequence of intensified proliferation and/or reduced apoptosis in the epithelium cells of breast. STAT1 is involved in both procedures due to its capacity for inducing the genes expression inhibiting proliferation of cell and/or inducing apoptosis (Kim and Lee 2007). The role of STAT1 transcription in the apoptosis of the mammary gland was inferred by the Schreiber group's findings in which the transcriptionally inactive mutant form of STAT1 (Y701F) could not induce apoptosis (Chan et al. 2012). Yet, no signs of apoptosis or cell death were observed in the experimental system of the Sexl group. Contrarily, they observed improved proliferation in vivo in tumorigenic and non-tumorigenic breast epithelium of $STAT1^{-/-}$ cells (Schneckenleithner et al. 2011). Additional proof for STAT1's role as the fundamental regulator of the proliferation of breast epithelium cells was derived from three-dimensional (3D) culture researches, which was employed by the Sexl group to make a comparison of mammospheres formation from primary epithelium cells of breast in virgin $STAT1^{-/-}$ compared with $STAT1^{+/+}$ mice. It was reported that remarkably thicker mammosphere layers were formed by $STAT1^{-/-}$ cells with an elevated rate of proliferation, and, in some cases, they could fill in the acini lumen (Schneckenleithner et al. 2011). The usefulness of the 3D culture approach may be proved in the study of the MIN formation mechanisms in STAT1-deficient cells of epithelium.

The STAT1 antitumor effect is correlated with its potential to induce the tumors' immune surveillance (Dunn et al. 2006); thereby, Koromilas et al. additionally investigated the STAT1's contribution in the stromal compartment to inhibit tumorigenesis of breast meditated by Neu NDL2–5. Thus, Neu NDL2–5 $STAT1^{-/-}$ and $STAT1^{+/+-}$ isolated tumor cells of breast of mice and underwent evaluations of orthotopic transplantation in the syngeneic $STAT1^{-/-}$ or $STAT1^{+/+}$ mice. It was shown that the transplanted tumors' growth was not remarkably different between tumor cells of $STAT1^{-/-}$ and $STAT1^{+/+}$ in the $STAT1^{+/+}$ recipient mice that were observed for nearly 9 weeks (Raven et al. 2011). Yet, tumor development of Neu NDL2–5 $STAT1^{-/-}$ transplanted cells of breast tumor during the same period was remarkably promoted in comparison with $STAT1^{+/+}$ cells of breast tumor in the $STAT1^{-/-}$ recipient mice (Raven et al. 2011). Breast tumor

histopathologic analysis revealed phenotypes of two tumor: a typical solid, nodular ErbB2/Neu-type tumor and a glandular papillary tumor, showing no difference between $STAT1^{-/-}$ and $STAT1^{+/+}$ mice (Raven et al. 2011). These data showed the tumor-site specific presence and effects of stroma in suppressing tumorigenesis of breast meditated Neu NDL2–5 by due to STAT1.

Additional evidence for a fundamental STAT1'role in the tumoral microenvironment was provided by the Sexl and Schreiber groups studies (Schneckenleithner et al. 2011; Chan et al. 2012). The Schreiber group described the selective STAT1 downregulation in a large cohort study of patients according to histochemistry analysis on biopsies of tumor (Chan et al. 2012). Downregulation of STAT1 was highly noticeable in cells of the tumor in comparison with the infiltrating lymphocytes and stromal surrounding. The STAT1 expression in the infiltrating lymphocytes and stroma of tumor may clarify why many studies have shown high levels of STAT1 in the samples of mammary cancer under experimental conditions, not allowing cells of tumor stroma separation from the other cells of tumor, including microarrays or western blotting of total tumors. These data underlined the fundamental role of the development of tumor microenvironment and the request for analysis of resolution of single cell to show the protein expression to a distinctive compartment of cells. It was shown by the Sexl group that deficiency of STAT1 serves to remarkably improve the frequency of spontaneous development of breast tumors in mice (Schneckenleithner et al. 2011). Also, the same group showed the dual STAT1 activity in suppression of the formation of breast tumor: however, STAT1 preserved the surveillance of tumor dependent to cytotoxic T lymphocyte (CTL) and was needed for full-fledged T cell cytotoxicity; however, STAT1 inhibited the proliferation of cells in the epithelium cells of breast in a cell-autonomous way (Schneckenleithner et al. 2011). That the STAT1 can control the immune cells' activity plays a crucial role in tumorigenesis regulation; i.e., preserved CTL activation by STAT1 leads to the suppression of breast tumors. Contrarily, STAT1 loss in the leukemic cells induces the robust natural killer (NK) cells activation by downregulation of MHC1 molecules (Kovacic et al. 2006).

To comprehend the STAT1's antitumor mechanism, Koromilas et al. investigated the phosphorylation of STAT1 regulation in signaling meditated by ErbB2. They showed that phosphorylation of STAT1 Y701 is reduced in ErbB2-positive cells of human mammary tumor following treatment with ErbB2 drug inhibitors, though not following treating with Src or JAKs inhibitors (Raven et al. 2011). Their findings showed that the activation of ErbB2 leads to elevated STAT1 Y701 phosphorylation through the mediation of signaling pathways, a notion further confirmed in STAT1's transient expression assays as well as an activated Neu form (Raven et al. 2011). They showed that STAT1 is an EGFR's substrate in vitro, though it is still unclear whether elevated phosphorylation of STAT1 Y701 in tumor cells of breast is directly affected by ErbB2 (Raven et al. 2011). Recent findings further supported ErbB2 and STAT1 functional interactions demonstrating the induced STAT1 expression via ErbB2 at the level of transcription via activation of STAT3 (Han et al. 2013).

Attempting to clarify the downstream activity of STAT1 of ErbB2/Neu, the Koromilas group investigated the impacts of phosphorylation of the STAT1's

defective mutants, STAT1S727A and STAT1Y701F, in transformation meditated by ErbB2/Neu. It was shown that the STAT1 phosphorylation mutants' expression in assays of xenograft tumor of SCID mice compromised by immune efficiently impaired the Neu NDL2–5 transforming properties in $p53^{-/-}STAT1^{-/-}$ mouse embryonic fibroblasts (MEFs) (Raven et al. 2011). This conclusion proposed that STAT1 is able to inhibit mediated tumorigenesis meditated by ErbB2/Neu regardless of phosphorylation at either S727 or Y701. Since STAT1 and ErbB2/Neu signaling networks depend on context, it is still probable that the phosphorylated STAT1 has another activity in MEFs compared with its activity in epithelium cells of the breast. The study of transformation mediated by ErbB2/Neu was conducted in MEFs deficient of p53 (Raven et al. 2011). Thus, it is crucial to assess the impacts of STAT1 phosphorylation mutants in Neu NDL2–5-transformed $STAT1^{-/-}$ epithelium cells of the breast with wild-type p53 (Ursini-Siegel 2011). A fascinating study performed by the Koromilas group showed that the Neu NDL2–5 at tyrosine residues in addition to Y701 in transiently transfected cells can phosphorylate STAT1 (Raven et al. 2011). Up to now, little information is available regarding STAT1 regulation via phosphorylation at sites other than S727 and Y701. Earlier researches by the Maniatis group revealed the STAT1 phosphorylation via IKKε at S708, an alternation contributing to the promotion of transcription of a certain set of IFN-inducible genes (Tenoever et al. 2007). It is still probable that ErbB2 signaling results in the STAT1 phosphorylation at novel sites contributing to its antitumor activities in the breast gland.

The Sexl group suggested that interferon regulatory factor 1 (IRF-1) mediates the STAT1's inhibitory effects on ER-positive growth of epithelium of the breast (Schneckenleithner et al. 2011). Considering that STAT1 induces IRF-1 at the level of transcription, this description is in line with the results of the Schreiber group indicating that inhibition of tumor growth of ER^+ mouse needs a transcriptionally active STAT1 (Chan et al. 2012). IRF1 role was demonstrated experimentally by the remarkable downregulation of IRF-1 in tumors with deficiency in STAT1 as well as the structural similarities found between $IRF1^{-/-}$ and $STAT1^{-/-}$ breast tissues based on the 3D mammospheres (Schneckenleithner et al. 2011). Studies that IRF1 is often heterozygous in tissue of human mammary tumor, as well as its downregulated expression in high-grade mammary cancers, further supports IRF1's potential role in breast cancer (Schneckenleithner et al. 2011). Approval of the IRF1 activity downstream of STAT1 requires using $STAT1^{-/-}IRF1^{-/-}$ mice to assess the effect of the STAT1-IRF1 axis deficiency on the development of the mammary tumor. Moreover, it is interesting to describe whether downregulation of IRF1 in human mammary tumors is correlated with reduced expression of STAT1 or expression of a transcriptionally inactive STAT1.

2.3 STAT2 and Breast Cancer

Although the human breast tumors' percentage expressing STAT2 has not been determined, STAT2 has been found in MCF-7 cells, a cell line in ER+ human breast cancer (Schaber et al. 1998; Uluer et al. 2012). Uluer et al. revealed prominent

cytoplasmic localization, and Schaber et al. reported the presence of STAT1-STAT2 heteromeric complexes following stimulation of type-I interferon.

2.4 STAT3 and Breast Cancer

Quantitative image analysis was used to look at tyrosine-phosphorylated STAT3 expression in 45 Stage III invasive breast tumors (Diaz et al. 2006). Phospho-STAT3 expression was found in 52% of the tumors, and it was linked to HER2 positive. Lower phospho-STAT3 levels were linked to a full pathogenic reaction. In a tissue microarray investigation of 346 node-negative breast tumors, STAT3 was found in the cytoplasm and nucleus of 69% and 23% of the tumors, respectively, while phospho-STAT3 (Tyr705) was found in the cytoplasm and nucleus of 23% and 44% of the malignancies, respectively (Dolled-Filhart et al. 2003). The nuclear STAT3 presence staining was linked to better survival in this study.

Phosphorylated STAT3 levels were measured in 923 specimens using a SAMBA 2050 automated equipment, which found expression of phosph-STAT3 in 34% of the samples and linked it to a worse prognosis (Charpin et al. 2009). In a study on 571 breast tumors, STAT3 expression was detected in 41% of the tumors, with no correlation to status of ER or prognosis (Yamashita et al. 2006). STAT3 ser727 phosphorylation was found to be lower in ER+ tumors than in ER malignancies in one research of 68 infiltrating ductal carcinomas, with equal expression level in HER2-positive and HER2-negative tumors (Yeh et al. 2006). In conclusion, these studies show that STAT3 is expressed in a large amount of all breast cancer subtypes; however, the prognostic importance and levels of STAT3 in various breast cancer subtypes vary.

The STAT3 signaling pathway, activated through growth factors or cytokines binding to their corresponding cell surface receptors, has been widely investigated (Qin et al. 2019; Ma et al. 2020). A short summary of the STAT3 signaling, STAT3 non-receptor tyrosine kinases, and intrinsic coactivators and inhibitors is provided in the following. Cytokine receptors when overexpressed, such as interleukin-10 receptor (IL-10R) and interleukin-6 receptor (IL-6R), as well as hyperactive growth factor receptors, such as insulin-like growth factor receptor (IGFR), fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR), always stimulate the phosphorylation cascade of tyrosine via ligands binding to these receptors (Yu et al. 2014). Once ligands bind to their cell surface receptors, mentioned receptors can form dimers and recruit JAKs and glycoprotein 130 (gp130), phosphorylating and activating JAKs (Garbers et al. 2015).

In contrast, active JAKs phosphorylate these receptors' cytoplasmic tyrosine residues, which subsequently bind with STAT3's SH2 domain, leading to JAK phosphorylation of STAT3 at Tyr705 (Johnson et al. 2018). Several non-receptor tyrosine kinases, including Src and Abl, may also phosphorylate and activate STAT3 (Karras et al. 1997). Through contact between their phosphorylated Tyr705 site and the SH2 domain, phosphorylated STAT3 (pSTAT3) can form a homodimer, prompting the difference of dimers of STAT3 in cell surface receptors and their

translocation from the cytoplasm into the nucleus (Yu et al. 2007; Wang et al. 2018). The nuclear STAT3 binds to particular sequences of DNA and promotes the gene transcription that governs different cancer cells' phenotypes with the aid of coactivator proteins such as apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE/Ref-1), NCOA/SRC1a, and CREB binding protein (CBP)/p300 (Yu et al. 2014; Guanizo et al. 2018).

STAT3's oncogenic ability in regulation of genes expression involved in proliferation of cancer cell, invasion, chemoresistance, angiogenesis, migration, stem cell self-renewal and maintenance, anti-apoptosis, immunological suppression, and autophagy has been extensively acknowledged (Yu et al. 2014; Guanizo et al. 2018). Notably, STAT3 is overexpressed and constitutively active in TNBC, which is linked to the disease's genesis, development, metastasis, chemotherapy resistance, and poor prognosis (Sirkisoon et al. 2018). STAT3 promotes the aggressiveness of TNBC by physically interacting with and functionally cooperating with other oncogenic and transcriptional factors, such as GLI1, to induce the expression of cancer-related genes (Sirkisoon et al. 2018). A decrease in an intrinsic inhibitor of STAT3 transcription, the gene correlated with retinoic-interferon-induced mortality 19 (GRIM-19), was also discovered in TNBC in recent research (Zhou et al. 2013). TCPTP, comprising the two splice variants TC48 and TC45, is likewise downregulated in TNBC cells in vitro and in vivo, contributing to STAT3 signaling activation (Shields et al. 2013). Indeed, STAT3 can localize in the mitochondria, where it is known as mitoSTAT3 and affects mitochondrial processes such as the chain of electron transport, ATP production, homeostasis of calcium, and accumulation of ROS (Wegrzyn et al. 2009; Yang and Rincon 2016). Furthermore, mitoSTAT3 has been demonstrated to enhance breast cancer cell proliferation, with Serine 727 phosphorylation playing a key role (Zhang et al. 2013). TNBC has a high level of acetylated STAT3, which causes inactivation and methylation of promoters of tumor suppressor gene, according to recent research (Lee et al. 2012). Importantly, mutating STAT3 at Lys685 or lowering acetylation of STAT3 with resveratrol may cause estrogen receptor gene demethylation and activation, making TNBC cells more sensitive to antiestrogens.

2.5 STAT4 and Breast Cancer

STAT4 expression has been observed both in MCF-7 cells (Liu et al. 2012; Uluer et al. 2012) and breast cancer cells. High expression of IL-12/STAT4 axis molecules including the IL-12 receptor genes and STAT4 were reported to significantly increase survival of breast cancer patients, particularly in the most aggressive subtypes including HER-2+, the luminal B (LumB), and basal like (Núñez-Marrero 2019). According to the profile integrative molecule, variations in STAT4 signaling are involved in the difference between low and high mammographic density (Kristensen et al. 2012). Because the researchers investigated the whole tissue, it was unclear if the differences in expression of STAT4 were observed in epithelium or stroma.

2.6 STAT5a/b and Breast Cancer

The role of STAT5 has been widely investigated in breast cancer (Tan and Nevalainen 2008). In 76% of 78 human breast adenocarcinomas, nuclear-localized tyrosine-phosphorylated STAT5a was identified (Cotarla et al. 2004). High levels of nuclear-localized p27 histological differentiation and expression were linked to the presence of nuclear-localized STAT5a. On tissue arrays of roughly 160, 683 and 443 specimens of breast cancer, expression of tyrosine-phosphorylated STAT5a/b was analyzed (Nevalainen et al. 2004). STAT5a/b activation was found in 19% of node-positive breast and 32% of node-negative breast cancers, and it was linked to a better prognosis. Another study found that 34% of 517 breast tumors tested positive for STAT5a/b, with favorable relationships between STAT5a/b with histologic grade, PR, and ER (Yamashita et al. 2006). Recent research on nuclear-localized phosphorylated STAT5a/b showed that 42% of tumors had increased levels of staining for STAT5a/b as opposed to low levels, and that these increased levels were associated with a better prognosis (Peck et al. 2011). Altogether, the findings show that nuclear-localized phosphorylated STAT5a/b is expressed in a considerable breast tumors' proportion and that this is a good prognostic signal in general.

2.7 STAT6 and Breast Cancer

Expression of STAT6 is now mainly characterized in cell lines of human breast cancer, but not in vast series of human breast cancer tissue, as is the case with STAT2 and STAT4. The ER+ cell line of breast cancer MCF-7 and ZR-75-1 has been found to express STAT6 (Gooch et al. 2002; Zhang et al. 2008; Godinho et al. 2012; van Agthoven et al. 2012).

2.8 ER+ Breast Cancer Subtypes and STAT Signaling

In ER+ human breast malignancies, STAT 1, STAT 3, and STAT 5a/b are all expressed, while STAT 2, STAT 4, and STAT 6 are expressed in cell lines of ER+ breast cancer, as shown previously. Except one study (Choi et al. 2013), several investigations so far have looked at ER+ breast cancer as a whole rather than dividing the samples into luminal A and B subtypes. Two researches found a connection between expression of STAT1 and positivity of ER (Magkou et al. 2012; Choi et al. 2013), whereas another study found that expression levels of STAT1 were decreased in ER+ malignancies than in ER− cancers (Chan et al. 2012). In 346 ER+ breast tumors, the effect of STAT3 and STAT5a/b expression on tamoxifen responsiveness was investigated (Yamashita et al. 2006). STAT5a/b-positive tumors had a better prognosis, according to the study, with an elevated rate of response to treatment with endocrine and longer life following relapse. STAT3 expression, on the other hand, did not appear to be linked to treatment response or prognosis. In two separate cohorts ($n = 221$ and $n = 97$) receiving

antihormonal medication with or without adjuvant chemotherapy, the positive connection between expression of STAT5 and enhanced response to treatment with endocrine was established again (Peck et al. 2011). The antihormonal medicine type used in the first group was not specified, whereas tamoxifen was used in the second. In a small group of 16 patients, however, STAT5a/b expression levels did not have any prediction of response to a 6-month course of exemestane, a steroidal irreversible aromatase inhibitor (Yamashita et al. 2009). In fact, STAT5 expression increased after therapy in our study. The apparent consistency of the association between increased levels of expression of STAT5a/b nuclear and tamoxifen responsiveness justifies more investigation of the mechanism at work, especially since some tissue culture data contradicts with these clinical findings (Riggins et al. 2006). The activation of STAT5a/b and other activated STATs in relation to expression patterns of gene connected to the luminal A and B subtypes may give an overview of the differing prognostic results and responses to treatment with endocrine in ER+ breast tumors (Chia et al. 2012; Geyer et al. 2012).

2.9 HER2/Neu + Breast Cancer and STAT Signaling

Expression of STAT3 has been linked to HER2 overexpression (Diaz et al. 2006). In HER2 breast cancer stem cells, a HER2-STAT3 signaling network has been discovered (Duru et al. 2012), and therapy targeted HER2 lowers STAT3 gastric cancer phosphorylation (Kim et al. 2008). In cell lines, STAT3 and HER2 mechanistic linkages have leptin-induced elevates in expression of HER2 via STAT3 signaling (Giordano et al. 2013) and STAT1 expression improvement by STAT3 and HER2 (Han et al. 2013). The fact that only a small percentage of HER2-amplified breast tumors express STAT1 (Choi et al. 2013) could mean that this pathway is only active in a subset of human HER2-amplified breast cancers or that it is highly relevant in vitro than in vivo. Further research into the involvement of STAT in HER2-amplified breast cancers, particularly STAT3 and STAT5a/b, can be useful to determine if STAT have a role in prognosis or responsiveness to therapy in HER2-amplified breast cancers.

2.10 TNBC and STAT Signaling

According to one study, the fraction of TNBCs with expression of STAT1 in cancer cells is likely to be low, with the expression occurring more commonly in cells of stroma (Choi et al. 2013). On the other hand, two researches recommended that the expression of STAT1 in TNBC is associated with a better prognosis (Charpin et al. 2009; Yau et al. 2010); however, one study looked at STAT1 levels in TNBC cancerous cells and found a link between increased expression levels and node-positive disease (Greenwood et al. 2012). They also found that STAT2 and STAT3 were expressed at similar levels in node-negative and node-positive breast tumors, however, not in TNBCs. In vitro studies of TNBC cells show that decreasing the acetylation of STAT3 via resveratrol results in elevated ER expression and

tamoxifen sensitivity's emergence (Lee et al. 2012) and that decreasing activation of STAT3 via administration of penta-*O*-galloyl-β-D-glucose (a herbal compound) decreases growth and metastases of xenograft (Lee et al. 2011), and this decrease works in tandem with metformin to slow the proliferation of cell lines of TNBC (Deng et al. 2012). After addressing the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway, JAK2/STAT5 activation can reduce the effect of this therapeutic introduction (Britschgi et al. 2012). TNBC therapy may need dual targeting of JAK2/STAT5 and PI3K/mTOR. Because gene array profiling can further classify TNBCs into subtypes (Lehmann et al. 2011), more delineation of expression patterns of STAT within these subtypes is needed before strong conclusions can be reached about the effect of various STAT proteins on prognosis and treatment response.

3 STAT Proteins: The Promising Targets in the Treatment of Breast Cancer

The etiology of various ranges of solid malignancies, including ovarian, breast, and prostate cancer, as well as malignant blood neoplasms, including myeloma and acute lymphoblastic leukemia, has been linked to the constitutive STAT3 and/or STAT5 activation. However, because abnormal activation of STAT3 was discovered more regularly than dysregulated STAT5, STAT5 has gotten less attention than STAT3, which is a therapeutic aim (Loh et al. 2019). Consequently, the focus of this chapter is on STAT3 as a possible therapeutic aim for breast cancer.

A member of the tyrphostin family of the inhibitors of tyrosine kinases small molecule like JAK2, AG490, demonstrated an ability to block the JAK2 tyrosine phosphorylation, which led to the STAT3 tyrosine phosphorylation inhibition. In MDA-MB 231 human breast cancer cell line, AG490 suppressed the phospho-STAT3 (Tyr705) expression as well as survivin, an anti-apoptotic protein, therefore blocking proliferation of cancer cells via induction of cell death (Yu and Deng 2006). Moreover, AG490 in MDA-MB-231 cells significantly inhibited cell adhesion, invasion, as well as metastasis (Chen et al. 2008). In another in vitro study, a modified structure of AG490, WP1066, overcame the TNBC drug resistance to Doxorubicin (Cheng et al. 2018). WP1066 is presently going through phase 1 RCTs in patients with melanoma which has spread to the brain or patients with malignant glioma which has come back to investigate the safety and tolerability and detect the maximum tolerated dose (NCT01904123). Tofacitinib is a targeted inhibitor of JAK3 and JAK1 and also has a partial inhibitory effect on JAK2 (Schwartz et al. 2016). An FDA-approved drug Tofacitinib is being used as a therapeutic medication in clinics for neoplastic and diseases inflammatory like ulcerative colitis, rheumatoid arthritis, and psoriasis (Dhillon 2017). It has been found through a phospho-proteome analysis that JAK/STAT signaling pathway exerts a role in the chemotherapy resistance of MCF7 breast cancer cells. Treatment with Tofacitinib in chemoresistant MCF7 cells was shown to increase chemosensitivity, resulting in higher rates of cancer cell death. This suggests the potential of Tofacitinib as a novel

therapeutic agent to combat breast cancer cells chemoresistance (Nascimento et al. 2017). Studies have also shown that Tofacitinib could be of high efficacy in the anti-drug antibody immune responses reduction, often occurring when immunotoxins are given to study participants or testing animals with a healthy immune system (Onda et al. 2014).

Furthermore, Tofacitinib treatment in mice with MDA-MB-468 xenografts enhanced the antibody-based agent's delivery to tumor cells via cytokine signaling reducing as well as suppressing the recruitment of malignancy-related inflammatory cells. Accordingly, an increase in immunotoxin efficacy was reported in xenograft mouse models of TNBC (Simon et al. 2019). It has been also reported that, as a single agent, Tofacitinib caused no remarkable adverse effects on solid tumors in RCTs. The limited Tofacitinib effectiveness could be due to the RCT designs (only late-stage patients being chosen and most of whom previously failed to several medical interventions). This can also be explained by the fact that JAKs aberrant activation is not completely identified in solid tumors yet (Huynh et al. 2017). Therefore, Tofacitinib could still be considered as a promising sensitizer when used combined with other chemotherapy medications.

Small molecules, peptides, derivatives of natural product, and peptidomimetic molecules, oligonucleotides, and other inhibitors of STAT3 can be divided into many various types based on their chemical structures: small molecules, oligonucleotides, peptidomimetic molecules, natural product derivatives, and so on. In addition, inhibitors targeting the SH2 domain, as well as those aiming the DNA binding domain or other domains, can be classified into a few different classes based on the aimed STAT3's structure or region (Chai et al. 2016). From the basic scaffold of chemicals, many inhibitory peptidomimetic molecules and peptides of STAT3 have been produced. The first peptide used was Pro-pTyr-Leu-Lys-Thr-Lys, which can bind to natural C-terminal STAT3-SH2 domain and hence precludes hetero- or homodimerization with phosphorylated STAT3 monomer (Turkson et al. 2001). Peptidomimetics outperform peptides in terms of pharmacokinetic characteristics. Even inhibitors based on peptides can have a high affinity for STAT3, yet, because of their negative charge on the phosphotyrosine group and peptidic structure, majority of them have deficiency in permeability of cell. As a result, several novel peptidomimetic compounds have been created. CJ 1383, a new cell-permeable inhibitor, was found to inhibit STAT3 effectively, leading to MDA-MB-468 cell line death of breast in a dose- and time-dependent way (Chen et al. 2010). Small molecule drugs and STAT3-SH2 domain interaction had potent inhibitory impacts on STAT3 dimerization, translocation of nucleus, and activation of transcription, similar to peptidomimetics. Static is the first non-peptide small molecule produced to be a STAT3 inhibitor. It suppressed the development and survival of various cell lines of breast cancer, including MDAMB-231 and MDA-MB-435, via selectively disrupting STAT3 dimerization over other STAT members and in an efficient way interacting with the STAT3 SH2-domain (Song et al. 2005). Noori et al. showed that naringenin could enhance cyclophosphamide's anticancer effect against MDA-MB-231 breast cancer cells through targeting the STAT3 signaling pathway. They reported that naringenin could trigger apoptosis

and significantly decrease cell viability. The coadministration of naringenin with cyclophosphamide enhanced its antitumor activity. Naringenin was also shown to decrease the expression of Bcl-2 and upregulate the expression of BAX. Naringenin activated caspases 3 and 9; this was also augmented via cyclophosphamide (Noori et al. 2020). LLL12 and LLY17 are two structural analogs of STA-21 that have been produced. In numerous cell lines of human cancer, such as U87, SK-BR-3, U373, HPAC, and PANC1, they all demonstrated a high specificity for inhibiting Tyr705 phosphorylation of STAT3 (84). In vitro cellular and in vivo animal models, cell-permeable compounds LLY17 and XZH-5 displayed efficacy on STAT3, as shown by the reduction of STAT3-induced biological procedures including migration, survival, and tumor growth (Linher-Melville et al. 2019; Liang 2020). Additional drugs targeting the SH2 domain have been created and demonstrated to decrease STAT3. In the murine model of bone pain induced by human breast cancer, for example, DR-1-55 suppresses the inflammatory cytokines' release including IL-6 and IL-1 (Linher-Melville et al. 2019). By targeting the SH2 domain, 6Br-6a revealed inhibitory impacts on volume of tumor in the MDA-MB-231 xenograft mouse model (Liu et al. 2020).

Finally, ODZ10117(3-(2,4-dichloro-phenoxy methyl)-5-trichloromethyl-[1, 2, 4] oxadiazole) is a recently produced small-molecule of STAT3 inhibitor found by structure-based computational database screening to recognize compounds aiming the STAT3 SH2 domain. By participating for the SH2 domain, it effectively blocked STAT3 phosphorylation. Breast cancers in vitro and in vivo models, ODZ10117 inhibited tumor growth, tumor migration and tumor invasion, tumor survival, and lung metastasis, indicating its effectiveness in inhibition of STAT3 (Kim et al. 2019). Some natural chemical entities have also been recognized as functional inhibitors that compete with the SH2 domain to reduce STAT3 activation. In cultured cell lines of breast cancer, curcumin, a component in turmeric, can selectively bound to the SH2 domain and abrogate phosphorylation of STAT3 phosphorylation, causing prevention of dimerization of STAT3 and inactivating STAT3 downstream cascade (Lin et al. 2009). The resveratrol-caffeic acid hybrid molecule 7d, on the other hand, is the first synthetic inhibitor to cause inhibition of acetylation and phosphorylation of STAT3. In breast cancer 4 T1 xenograft models, this novel derivative severely inhibited proliferation of cell via triggering growth of tumor and cell death in vitro (Li et al. 2016). GLG-302 (S3I-201, NSC 74859) is another novel STAT3 inhibitor. Its salicylic acid moiety binds to the STAT3-SH2 domain's pTyr binding site. In a mouse model, it exhibited activity against breast cancer cells and the ability to prevent receptor-negative mammary tumors and estrogen receptor-positive (Siddiquee et al. 2007). Following a computer-aided lead optimization, further STAT3-SH2 dimerization inhibitors were created using in silico site-directed fragment-based drug design. The selective STAT3 inhibitor C188–9 (STAT3 inhibitor XIII, TTI-101) binds to the STAT3-SH2 domain. C188–9 therapy inhibited STAT3 and lowered the programmed death-ligand 1 (PD-L1) expression in 4 T1 breast cancer mice, resulting in tumor growth and metastasis being limited (Zerdes et al. 2019). C188–9, in particular, is currently being tested in a phase I clinical trial for patients with advanced cancer, particularly those with breast cancer (NCT

03195699). All of these data point to the SH2 domain of STAT3 as a promising aim for novel breast cancer treatment approaches. Another type of STAT3 inhibitor targets STAT3's N-terminal region, also known as the transcriptional activation domain, which is necessary for binding of DNA, interaction of protein-protein, nuclear translocation, and activation of transcription.

ST3-H2A2 is a highly specific peptide inhibitor of the STAT3 N-terminal domain that efficiently inhibits transcription and dimerization effects of STAT3. ST3-H2A2 was found to cause cell death in cells of breast cancer MCF-7 and MDA-MB-231 however had no influence on the normal mammary epithelial cells of epithelium's survival MCF-10A, implying that it is cancer cell specific (Timofeeva et al. 2007). Platinum compounds including IS3 295, CPA-1, and CPA-7 have also been demonstrated to inhibit activated STAT3-mediated anti-apoptosis and progression of cell cycle in numerous cell lines of breast cancer by disrupting STAT3's capacity to bind its DNA response element (Turkson et al. 2004; Turkson et al. 2005). Furthermore, STAT3 N-terminal domain inhibitors based on nucleotides, such as STAT3-G-Quartet and STAT3-siRNA, showed significant inhibitory impacts on activity of STAT3 in a variety of in vitro cellular types. These findings imply that aiming the STAT3 N-terminal region may have therapeutic promise in the breast cancer pathogenesis caused by constitutively active STAT3 (Jing et al. 2004; Kunigal et al. 2009). Finally, pyrimethamine (also known as PYR or 2,4-diamino-5-p-chlorophenyl-6-ethyl-pyrimidine) is approved by FDA, an antimicrobial medication that has been introduced to treat toxoplasmosis and malaria. PYR therapy had anticancer and immune-stimulatory benefits in breast cancer mouse models, as well as significant inhibitory impacts on transcriptional activity of STAT3 (Legorreta-Herrera et al. 2010; Takakura et al. 2011; Khan et al. 2018). As a result, it's important to consider PYR's efficacy in the breast cancer treatment. BPMB (5,5′-(pentane-1,5′-diyl) bis (2-methyl-1,4 benzoquinone) was discovered to be another STAT3 transcriptional inhibitor. BPMB inhibited STAT3 transcription activation almost completely without changing its phosphorylation or translocation of nucleus. The mechanism of BPMB was discovered to be inactivation of the STAT3 complex via linker domain acylation (Koseki et al. 2019).

4 Conclusion

In both hematologic malignancies and solid tumors, STAT signaling was linked to survival, therapy resistance, metastasis, growth, and progression of cancer. STAT3 and STAT5 seem to play the most important roles in biology of cancer, whereas STAT6, STAT4, STAT2, and STAT1 play a smaller part. However, rather than being caused by a single dominating family member, their effects are currently thought to be caused by transcriptional changes mediated via a network of subtle and complicated interactions among distinct STAT proteins. The strength and length of the response to signal in a specific cell type are determined by the steady condition and signal-inducible function of the entire positive and negative regulators. JAK-STAT signaling can create a transcriptional result, and consequently the tissue

response and cell response are greatly influenced by a wide range of external and intrinsic stimuli. Because even small changes in STAT's cellular concentrations may have significant phenotypic repercussions, it's no surprise that their actions are context-dependent and occasionally contradictory. As a result, it's important to remember that screening for the function of a single STAT member may not be enough to associate with disease features, but the balance of (counteracting) family members may. This is important to remember when developing diagnostic or therapeutic tools related to STAT, and further studies of the mutual interactions among STAT proteins is required.

On the other hand, many resources have been devoted to the development of STAT3 inhibitors. Because of the varied activities of STAT3 in survival of cancerous cell, STAT3-targeted treatment may be predicted to generate excellent therapeutic results in breast cancer patients by a direct suppression of tumor cell growth. Furthermore, several oncogenes have been shown to upregulate the production of cytokines in order to JAK/STAT3 pathway activation, increasing tumor development. For instance, the oncogenes BCR-ABL tyrosine kinase and p21 Ras oncoprotein activate JAK/STAT signaling by increasing IL-6 production, indicating that STAT3 inhibitors may similarly limit the oncogenes' oncogenic effects on breast carcinogenesis (Yu et al. 2014). In breast cancer, JAK/STAT signaling has been shown to interact with other consistently activated signaling pathways such as PI3K/Akt (Kim et al. 2015). As a result, it is hypothesized that inhibiting JAK/STAT signaling might at least partially inhibit carcinogenesis induced by oncogenic pathways. More significantly, as previously indicated, preclinical research demonstrates that inhibiting STAT3 activity efficiently prevents and/or reverses breast cancer treatment resistance to several chemotherapeutic medicines like doxorubicin. Blocking JAK/STAT signaling has also been found to be crucial in radiotherapy-induced suppression of sphere-forming capacity and self-renewal of stem cells of breast cancer (Kaushik et al. 2017). STAT3 inhibitors can be utilized as a straight tumor suppressor and as a sensitizer in conjunction with different chemotherapy or radiation for breast cancer, according to this research. However, further study on the JAK/STAT pathway is needed to better understand its biology in diverse cellular contexts and with different kinds of breast cancer backgrounds. Simultaneously, more intensive study on JAK2 and STAT3's current small molecule inhibitors is needed to assess their in vivo efficacies, selectivities, bioavailabilities, and potential adverse health consequences. In a nutshell, the targeted treatment of breast cancer is still fraught with difficulties, but, as oncology research advances, new targets are discovered, and targeted drugs are evaluated; targeted treatment will usher breast cancer treatment into a new personalized healthcare era and will have greater effects on patients with breast cancer.

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