Chapter 10

Targeting the SH2 Domain of STAT3 Proteins in Breast Cancer Treatment

Busra Demir Cetinkaya[1]

Abstract

Stat proteins, transcription factors that convert extracellular stimuli into appropriate biological responses, are involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation. Irregular Stat activation is often associated with tumorigenesis. This situation has made the Stat pathway an interesting target for drug development studies in cancer treatment and has led to the development of various inhibitors targeting this pathway. Stat signal inhibitors are divided into two main groups as inhibitors with direct and indirect effects. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of the Stat3 protein; indirect inhibitors target upstream components of the Stat3 pathway, such as JAK2 and EGFR. It is known that Stat3 has a strong relationship with the formation of breast cancer and its permanent activation is most pronounced in breast cancer. In this study, primarily the components of the Stat signaling pathway, activation/inactivation and the functions of Stat3 were emphasized, the inhibitors that act by directly inhibiting the SH2 domain of Stat3 proteins in breast cancer cells were focused, and the results of the research examining the effects of these inhibitors on breast cancer cells were compiled.

Introduction

Signal converter and transcription activator (Stat) proteins are transcription factors that convert extracellular stimuli into appropriate biological responses (Catlett-Falcone, Dalton, & Jove, 1999). Stat proteins were first identified in 1994 as key proteins involved in cytokine signaling and interferon-related antiviral activity (Jr, Kerr, & Stark, 1994; Sadowski, Shuai, Jr, &

1 Assistant Professor; Department of Pharmaceutical Toxicology, Erzincan Binali Yıldırım University Faculty of Pharmacy, Erzincan, Turkey, e-mail address: ecz.busrademir@gmail.com, ORCID: 0000-0002-9926-107X

https://doi.org/10.58830/ozgur.pub128.c630

Gilman, 1993). Over time, they have been found to be involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation (Verhoeven et al., 2020). The Stat protein family consists of seven members, Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6 (Logotheti & Pützer, 2019). All Stat proteins consist of structurally and functionally conserved regions (Verhoeven et al., 2020). Although each is encoded by a separate gene (Furqan et al., 2013), they show high homology in their functional domains (Logotheti & Pützer, 2019). Structurally functional parts of Stat proteins; The N-terminal domain (ND), DNA binding domain, coiled-coil domain (CCD), Src-homology 2 (SH2) domain, and C-terminal transcriptional activation domain (TAD) each have an important function. ND mediates homo- and heterodimerization of Stat monomers with the highly conserved SH2 domain, which is the target of many Stat inhibitors. The DNA binding domain enables the DNA and Stat complex to form. CCD functions as a nuclear localization signal. The C-terminal transcription domain with highly conserved phosphorylated tyrosine (Y) and serine (S) residues recruits additional transcriptional activators and enhances the transcriptional activity of Stat (Verhoeven et al., 2020; Xin et al., 2020). Stat structural domains are shown in Figure 1.

Figure 1: Schematic representation of STAT structural domains

1. ACTIVATION of STAT SIGNALING PATHWAY

The activation process of Stats begins following the binding of cytokines, growth factors, and hormones to their receptors on the cell surface (Turkson & Jove, 2000). These receptors are receptor-related tyrosine kinases such as Janus kinase (JAK) or receptors with intrinsic tyrosine kinase activity such as Platelet-derived growth factor receptor (PDGFR), Epidermal growth factor recepor (EGFR), Fms-like tyrosine kinase 3 (FLT3). Stats are also known to be activated by constitutively active non-receptor protein tyrosine kinases (PTKs) such as c-Src Bcr-Abl and Breast tumor kinase (Brk) (Buettner, Mora, & Jove, 2002; Furqan et al., 2013; Weaver & Silva, 2007). With specific phosphorylation of Stat proteins by these tyrosine kinases (Furqan et al., 2013), the two Stat monomers form dimers via reciprocal phosphotyrosine-SH2 interactions, translocate to the nucleus, and bind to Stat-specific

DNA response elements of target genes to induce gene transcription (Turkson & Jove, 2000) and mediate processes related to cellular immunity, proliferation, apoptosis and differentiation (Logotheti & Pützer, 2019). However, the functionality of Stat proteins is not limited to forming dimers by phosphorylation. Unphosphorylated Stat dimers and tetramer/oligomer conformations also play a role in the functionality of some Stats (Moriggl et al., 2005; Park et al., 2016). Two nonphosphorylated Stat dimers can form tetramers with N-terminal oligomerization domains, stabilizing its binding to DNA (Y. Zhao et al., 2013). The regulation of the Stat signaling pathway is shown in Figure 2.

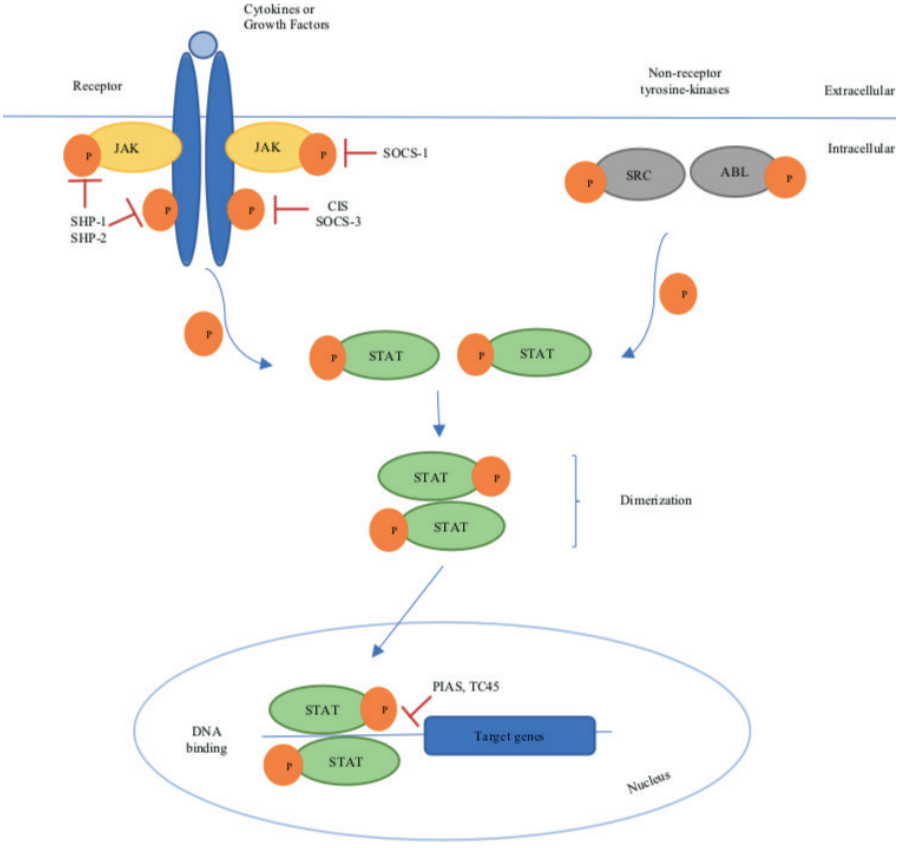

Figure 2: Regulation of STAT signaling pathway

2. NEGATIVE REGULATORS of STAT SIGNALING PATHWAY

The state of activation of stat proteins is transient. Activated Stat proteins are deactivated within hours and returned to the cytoplasm (Calò et al., 2003). Inactivation of Stat proteins is mediated by suppressor of cytokine signaling (SOCS), protein inhibitor of activated Stat (PIAS), and negative regulators including phosphatases (T. K. Kim & Maniatis, 1996). SOC is also known as JAK binding proteins (JABs) or cytokine-induced SH2 (CIS) proteins or Stat-induced Stat inhibitors (SSI) (Calò et al., 2003). The SOCS family consists of eight members, namely SOCS-1/JAB/SSI-1, SOCS-2, SOCS-3, SOCS-4, SOCS-5, SOCS-6, SOCS-7 and CIS (Lim & Cao, 2006). They inhibit Stat proteins by different mechanisms based on suppression of JAKs or competition for receptor binding (Lim & Cao, 2006). Namely; SOCS-1 suppresses JAK activity through direct interaction, while SOCS-3 inhibits Stat activation by first interacting with the phosphorylated receptor and then interacting with JAKs. CIS inhibits activation by competing with Stats for the same docking site on phosphorylated receptors (Lim & Cao, 2006). Some SOCS genes are transcriptionally regulated by Stats themselves, forming part of a classical negative feedback loop for cytokine signaling, indicating that Stats can negatively regulate their phosphorylation states (Calò et al., 2003; Desrivières et al., 2006). It is also known that SOCS proteins can cause receptor protein turnover mediated proteolytic degradation process via a ubiquitin-proteasome (Krebs & Hilton, 2001).

A second class of proteins that cause inactivation of Stat proteins is the protein inhibitor of activated Stat (PIAS). These proteins interact directly with Stat dimers in the nucleus to form protein complexes and block transcription. Namely; The resulting complexes cannot induce gene transcription because they do not bind to DNA or because nuclear co-repressor molecules are recruited into transcription complexes (Desrivières et al., 2006; Hodge, Hurt, & Farrar, 2005). The mammalian PIAS family, which consists of PIAS1, PIAS3, PIASy, PIASxa/ARIP3, and PIASxb/Miz1 have a certain degree of specificity towards Stat members; PIAS1 and PIASy are specific to Stat1, PIAS3 to Stat3 and Stat5, and PIASx to Stat4 (Liu & Shuai, 2003).

Another group of negative regulators of stat proteins is phosphatases. TC45, a nuclear tyrosine phosphatase in this group, deactivates phosphorylated Stats in the nucleus (Desrivières et al., 2006). There is evidence that TC45 is a related Stat phosphatase for Stat1 and Stat3, and has also been reported to

be involved in the regulation of cytoplasmic dephosphorylation of JAK1 and JAK3 (Ibarra-Sanchez et al., 2000; Simononic, Lee-Loy, Barder, Tremblay, & McGlade, 2002).

In addition to TC45, tyrosine phosphatases such as SHP1 and SHP2 are localized in the cytoplasm and have SH2 domains. These phosphatases disrupt JAK/Stat signaling by interacting with SH2 domains and phosphorylated tyrosine residues of JAKs and Stats (Desrivières et al., 2006). CD45, an active transmembrane molecule in hematopoietic cells, and PTP1B and TC-PTP phosphatases active in the cytoplasm of these cells are among the phosphatases responsible for the negative regulation of Stat proteins (Desrivières et al., 2006).

3. THE ROLE of STAT3 PROTEINS in the BREAST GLAND

Stat3 and Stat5 proteins are involved in fundamental changes in the mammary gland, including processes such as lactation and involution. Mice deficient in Stat3 have been found to die during early embryogenesis (Takeda et al., 1997). Mammary gland involution is a multi-step process in which the lactating gland morphologically returns to a state close to the pre-pregnancy state and a high degree of epithelial cell death and stromal rearrangement occurs (Groner & von Manstein, 2017; Stein, Salomonis, & Gusterson, 2007). Stat3 signaling induces epithelial cell death to clear differentiated milk-producing cells during involution (Groner & von Manstein, 2017). It has been shown that the disappearance of the lactation stimulus causes Stat3 phosphorylation to initiate involution, but the upregulation of pStat3 is not due to the decrease in lactogenic hormones, while Leukemia inhibitory factor (LIF) is the first activator of Stat3 during involution (Hughes & Watson, 2018; Kritikou et al., 2003; M. Li et al., 1997).

4. THE ROLE of STAT3 PROTEINS in BREAST CANCER

Stat3 is overactive in many types of cancer as a result of autocrine and paracrine stimulation by cytokines and growth factors such as interleukins (IL-6, IL-10, IL-12), interferons (IFNs), granulocyte colony stimulating factor (G-CSF or CSF3), prolactin (PRL), growth hormone (HGH), epidermal growth factor (EGF) , hepatocyte growth factor (HGF), essential fibroblast growth factor (FGF2), virus proteins (e.g., v-Src, v-Fps, v-Sis) or due to persistent activation via Intrinsic tyrosine kinase activities such as erb-b2 receptor tyrosine kinase 2 (ERBB2), epidermal growth factor receptor (EGFR), and hepatocyte growth factor receptor HGFR, non-receptor tyrosine kinases (such as c-Src and c-abl) or G protein-coupled receptor (Kortylewski, Jove, & Yu, 2005; Lim & Cao, 2006). It is known

that Stat3 is strongly associated with breast cancer formation and its permanent activation is most prominent in breast cancer (Groner & von Manstein, 2017).

Stat3 has been shown to be constitutively activated in approximately 70% of breast tumors (Alvarez et al., 2005). Although activated in all types of breast cancer, it has been most commonly associated with triple-negative breast cancer that lacks estrogen receptor (ER) or progesterone receptor (PR) expression and does not show Her2 amplification (L. Marotta et al., 2011; S.R Walker et al., 2009). It is an interesting paradox that Stat3 protein, which is involved in every stage of mammary gland development and has important roles in the basic changes in the mammary gland, has a strong relationship with mammary tumor formation (Groner & von Manstein, 2017; Sarah R. Walker, Xiang, & Frank, 2014). In normal breast cells, Stat3 is activated by Leukemia inhibitory factor (LIF) to promote involution with the abolition of the lactation stimulus (Hughes & Watson, 2018; Sarah R. Walker et al., 2014), whereas in many breast cancer cell lines, it is activated in an autocrine fashion by interleukin-6 (IL-6) produced by These cells (Lieblein et al., 2008; L. Marotta et al., 2011). Stat3, which is constantly in the activated state; can lead to malignant cell behavior by upregulating genes such as B-cell lymphoma 2 (BCL2), B-cell lymphoma-extra large (BCL-XL), Myeloid Cell Leukemia Sequence 1 (MCL1) that play a role in apoptosis, gene expression of cyclin D, the main target of transcriptional control of the cell cycle and other cell cycle and survival-related genes such as B -cell lymphoma 2 (BCL2), myc proto-oncogene (c-MYC). (Igelmann, Neubauer, & Ferbeyre, 2019). Recent studies have shown that Stat3 promotes the process of malignant transformation by activating genes involved in the Phosphoinositide 3-Kinase (PI3K) /AKT/ Mammalian Target of Rapamycin (mTOR) pathway, the Nuclear Factor Kappa-Light-Chain Enhancer of Activated B-Cells (NF-κB) pathway, and the cell cycle regulation pathway (Banerjee & Resat, 2016; Igelmann et al., 2019).

Oncostatin M (OSM), a member of the IL-6 cytokine family, can promote breast cancer progression by inducing upregulation of IL-6 and phosphorylation of stat3 (Ma, Qin, & Li, 2020; Tawara, Scott, Emathinger, Ide, et al., 2019; Tawara, Scott, Emathinger, Wolf, et al., 2019). In addition, while IL-35 inhibits conventional T (T-conv) cells and promotes breast cancer progression through Stat3 and Stat1 activation, IL-8 and growth-regulated oncogene (GRO) chemokines contribute to breast cancer progression by activating Stat3 (Hao). et al., 2018; Ma et al., 2020; Valeta-Magara et al., 2019). Stat3 is also known to contribute to breast cancer metastasis. Stat3 along with IL-6 has been shown to contribute to the malignant phenotype

of cancer cell by upregulating the expression of the EMT-inducing Twist, in part by promoting invasion and epithelial-mesenchymal transition (Lo et al., 2007; Sullivan et al., 2009; Sarah R. Walker). et al., 2014; Yadav, Kumar, Datta, Teknos, & Kumar, 2011). Stat3 is known to contribute to breast cancer metastasis by upregulating Matrix metallopeptidase 2 (MMP2), Matrix metallopeptidase 9 (MMP9), Snail, Slug, and vimentin (Kamran, Patil, & Gude, 2013; Z. Li et al., 2019; Ma et al., 2020). Stat3 is a protein that also affects the angiogenesis process of the tumor cell. It contributes to this process by up-regulating nodal factors of angiogenesis, particularly vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1 alpha (HIF-1α), and Matrix metalloproteinase-2 (MMP-2) (Kortylewski et al., 2005). Stat3 is also known to localize to mitochondria, and mito-Stat3 is known to regulate mitochondrial metabolism and mitochondrial gene expression (Chueh, Leong, & Yu, 2010; Igelmann et al., 2019; Macias, Rao, Carbajal, Kiguchi, & DiGiovanni). , 2014; Sala et al., 2019; Wegrzyn et al., 2009; Q. Zhang et al., 2013). Recent evidence suggests that Stat3 may promote survival of breast cancer cells through its effects on mitochondrial function (Gough et al., 2009). These proteins are known to cause drug resistance as well as processes such as tumor initiation, cell cycle, survival, metastasis and angiogenesis. Cancer stem cells (CSCs), also called tumor initiating cells (TICs), are a group of specialized cancer cells found in tumors that have the ability to self-renew and specifically produce a variety of tumor cells. These cells are considered to be responsible for recurrence and metastasis and resistance to treatment (Gibbs et al., 2005). In breast cancer, Stat3 has been shown to be essential for the viability of cancer stem cells (Hirsch, Iliopoulos, & Struhl, 2013), it has been reported that a non-CSC population can be transformed into a CSC-like population through OCT-4 regulation of the IL-6/JAK1/Stat3 signaling pathway (S. Y. Kim et al., 2013). In addition, it has been found that the JAK2/Stat3 signaling pathway in breast cancer increases chemoresistance by increasing carnitine palmitoyltransferase 1B (CPT1B) and fatty acid beta oxidation (FAO) (Wang et al., 2018). It has been determined that the Src/Stat3 signaling pathway is involved in multidrug resistance in triple negative breast cancer cells (Tzeng et al., 2018).

5. INHIBITON of STAT3 PROTEINS in BREAST CANCER TREATMENT

Stat signaling inhibitors are divided into two main groups as inhibitors that act directly and indirectly. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of Stat3 protein (McMurray, 2006; Xiong, Yang, Shen, Zhou, & Shen, 2014), indirect inhibitors target

upstream components of the Stat3 pathway such as JAK2 and epidermal growth factor receptor (EGFR) (Thilakasiria et al., 2021). In this review, we focused on inhibitors that act by directly inhibiting SH2 domain of Stat3 proteins in breast cancer cells.

5.1. SH2 Domain Inhibitors or Dimerization Inhibitors

The SH2 domain plays a critical role both in mediating the activation of Stat3 through its interaction with phosphorylated tyrosine residues on the cytoplasmic portion of the receptors, and in forming dimers of the two Stat3 monomers through reciprocal phosphotyrosine-SH2 interactions (Turkson & Jove, 2000; Xiong et al., 2014). Stat dimers migrate to the nucleus and mediate processes related to cellular immunity, proliferation, apoptosis and differentiation by binding to Stat-specific DNA response elements of target genes to induce gene transcription (Turkson & Jove, 2000) (Logotheti & Pützer, 2019). Inhibition of the SH2 domain suppresses the phosphorylation and activation of the Stat3 protein, resulting in inhibition of the cellular processes it mediates (Xiong et al., 2014). Considering the mentioned functions of the SH2 domain, molecules capable of blocking the SH2 domain of Stat3 have been evaluated for the treatment of different tumors (Tolomeo & Cascio, 2021). It can be said that the SH2 domain inhibits both the activation and dimerization of Stat3 proteins and is important in terms of creating an effective treatment approach for cancer treatment by preventing the dimerization of proteins that escape activation (Berg, 2008). Compounds that inhibit the SH2 domain of Stat3 proteins can be grouped as peptide and peptidomimetic and non-peptidic chemical inhibitors (new series of small molecules) considering their chemical structures. The schematic representation of SH2 domain inhibitors studied in breast cancer cells is shown in Figure 3.

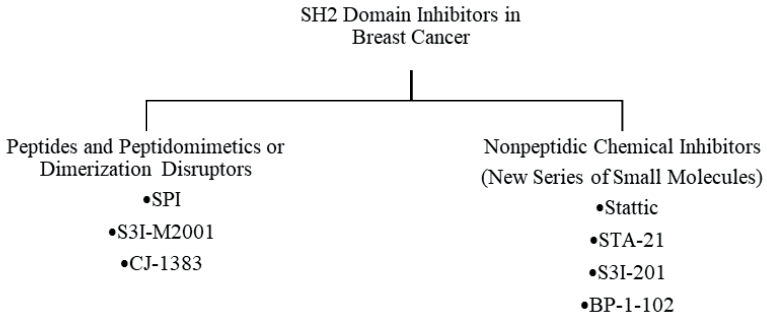

Figure 3: Schematic representation of SH2 domain inhibitors studied in breast cancer cells

5.1.1. Peptides and Peptidomimetics Targeting the STAT3 SH2 Domain in Breast Cancer

The SH2 domain inhibitor *SPI* is a 28-mer peptide derived from the SH2 domain of Stat3. It acts by strongly and selectively inhibiting the Stat3 SH2 domain interaction with the pTyr residue on the cytoplasmic tail of IL-6R (Xiong et al., 2014) (W. Zhao, Jaganathan, & Turkson, 2010). In a study by Zhao et al., SPI dose-dependently reduced cell viability and growth in MDA-MB-231 and MDA-MB-435 breast cancer cell lines with constitutively activated Stat3, and also induced apoptosis in MDA-MB-231 cells with constitutively activated Stat3 (W. Zhao et al., 2010).

Since peptidomimetics have better pharmacokinetic properties than peptides, peptidomimetic compounds have been developed by using the peptide XpYL compound as the basic scaffold (Furqan et al., 2013). *CJ-1383* is a cell permeable small molecule peptidomimetic targeting the SH2 domain (Chen et al., 2010; Thilakasiria et al., 2021). In the study of Chen et al., it was shown that CJ-1383 inhibited cellular Stat3 signaling and cell growth and induced apoptosis in a dose-dependent manner in MDA-MB-468 breast cancer cell line with constitutively activated Stat3 (Chen et al., 2010). *S3I-M2001* is an oxazole-based peptidomimetic of the Stat3 SH2 domain-binding phosphotyrosine peptide (K. A. Z. Siddiquee et al., 2007). The compound has been shown to inhibit Stat3-dependent transcription, transformation, survival and migration in both human and mouse cells by selectively disrupting Stat3 dimerization (K. A. Z. Siddiquee et al., 2007). In the study of Siddiquee et al., it was determined that S3I-M2001 inhibited the growth of human breast tumor xenografts (K. A. Z. Siddiquee et al., 2007).

5.1.2. Nonpeptidic Chemical Inhibitors Targeting the STAT3 SH2 Domain in Breast Cancer

The low cell penetration of phosphopeptides led to evaluation of the efficacy of a "new set of small molecules" for Stat3 SH2 domain inhibition (Xiong et al., 2014). These non-peptide molecules are cell permeable and have better physicochemical properties, unlike molecules derived from peptides or peptidomimetics (Yue & Turkson, 2009). Their mechanism of action is similar to peptidomimetics: by interacting with the Stat3-SH2 domain, they inhibit Stat3:Stat3 dimerization and thus nuclear translocation and transcriptional activity (Furqan et al., 2013).

Stattic (Stat three-inhibitory compound) was the first non-peptide inhibitor of Stat3 discovered (Schust, Sperl, Hollis, Mayer, & Berg, 2006).

Stattic inhibits the function of the SH2 domain of both unphosphorylated and phosphorylated Stat3, preventing Stat3 dimerization and binding to DNA (Berg, 2008) (Xiong et al., 2014). Stattic shows selective Stat3 inhibition; While it does not inhibit Stat1 and Stat5b in vitro, it has been shown to inhibit Stat3 (Berg, 2008). Stattic has been shown to induce apoptosis after permanently inhibiting the phosphorylation of Stat3 in breast cancer cell lines MDA-MB-231 and MDA-MB-435, which constitutively show Stat3 activation (Schust et al., 2006).*STA-21* is a natural deoxytetrangomycin, an angucycline antibiotic (Song, Wang, Wang, & Lin, 2005). It binds effectively to the SH2 domain of Stat3, effectively inhibiting Stat3 dimerization and abolishing its nuclear translocation. If we look at the results of the studies carried out in breast cancer cells in detail; STA-21 inhibited Stat3-dependent luciferase activity in MDA-MB-435s breast cancer cell line with constitutively activated Stat3 and showed high DNA binding activity in these cells. Another breast cancer cell line with constitutively active stat3 signaling, MDA-MB-468, also inhibited stat3 DNA binding activity and its donwstream antiapoptotic factors (Bcl-XL and cyclin D1), but phosphorylation of upstream regulators of Stat3 (P-JAK2, P-Src, P-EGFR) unaffected by STA-21.In the same study, the effects of STA-21 on cell growth and survival in breast cancer cell lines with constitutively active Stat3 activity as well as luciferase activity and DNA binding activity were investigated: MDA-MB-231, MDA-MB-435s, and MDA-MB-468 (that express persistently activated Stat3) significantly inhibited the survival of breast cancer cell lines, but showed minimal inhibitory effect on MCF-7 and MDA-MB-435 breast cancer cells (that have no constitutive Stat3 signaling) (Song et al., 2005). *S3I-201* (also known as NSC 74859) is a low molecular weight salicylic acid derivative and inhibits Stat3 dimerization by coupling the salicylic acid moiety with the pTyr binding site of the Stat3-SH2 domain (K. Siddiquee et al., 2007). S3I-201 significantly inhibited constitutive Stat3 activation in MDA-MB-231, MDA-MB-435 and MDA-MB-468 breast cancer cell lines with harbor constitutive Stat3 activation. In addition, treatment with S3I-201 in all three cell lines caused a decrease in the number of viable cells, while cell viability was not significantly affected in the MDA-MB-453 breast cancer cell line which do not harbor aberrant Stat3 activity. When it was examined whether the loss of cell viability caused by S3I-201 was mediated by apoptosis, it was determined that S3I-201 significantly induced apoptosis in the MDA-MB-435 breast cancer cell line with harbor constitutive Stat3 activation. In the same study, it was noted that S3I-201 caused a significant decrease in the expression of Stat3 target genes encoding Cyclin D1, Bcl-xL and Survivin in the MDA-MB-231 breast

cancer cell line with constitutive Stat3 activation. In the same study, it was determined that S3I-201 strongly inhibited tumor growth in human breast (MDA-MB-231) tumor-bearing mice (K. Siddiquee et al., 2007). *BP-102*, an analog of S3I-201, binds to the SH2 domain of Stat3, inhibiting Stat3-phospho-tyrosine (pTyr) peptide interactions and hence Stat3 activation by the same mechanism as S3I-201 (X. Zhang et al., 2012). In the study of Zhang et al., BP-1-102 suppressed cell proliferation, anchorage-dependent and independent growth, and colony numbers and also induced apoptosis in MDA-MB-231 breast cancer cells harboring aberrantly active Stat3. Overall, induction of Focal adhesion kinase (FAK) and paxillin phosphorylation and downregulation of E-cadherin are thought to contribute to Stat3-mediated malignant progression. Decreased phosphorylation of paxillin and FAK and increased expression of E-cadherin were seen in MDA-MB-231 cells treated with BP-1-102. To further investigate the effect of BP-1-102 on Stat3 cross-talks, the study examined its effect on the production of soluble factors by tumor cells: In culture medium from MDA-MB-231 cells treated with BP-1-102, granulocyte colony-stimulating factor (G-CSF), soluble intercellular adhesion molecule (sICAM) 1 and macrophage migration-inhibitory factor (MIF)/ glycosylation-inhibiting factor (GIF) levels were found to be lower, so it was concluded that BP-1-102 inhibited the production of soluble factors by tumor cells. Again in the same study, it was shown that BP-1-102 inhibited the growth of mouse xenografts of human breast (MDA-MB-231) tumor that harbor aberrantly active Stat3 as a result of intravenous and oral gavage administration without any significant changes in body weights or significant signs of toxicity such as loss of appetite, decreased activity or lethargy (X. Zhang et al., 2012).

6. CONCLUSION

Deregulated activation of the Stat pathway, which is involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation (Verhoeven et al., 2020), is frequently associated with tumorigenesis. It is known that Stat3 is strongly associated with breast cancer formation and its permanent activation is most prominent in breast cancer (Groner & von Manstein, 2017). Although activated in all types of breast cancer, it has been most associated with triple-negative breast cancer that lacks estrogen receptor (ER) or progesterone receptor (PR) expression and does not show Her2 amplification (L. L. C. Marotta et al., 2011; S.R Walker et al., 2009; Sarah R. Walker et al., 2014). The role of the Stat pathway in cancer development has made this pathway an interesting target for drug development in cancer

therapy and has led to the development of many inhibitors targeting this pathway. Stat signaling inhibitors are divided into two main groups as inhibitors that act directly and indirectly. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of Stat3 protein (McMurray, 2006; Xiong et al., 2014), indirect inhibitors target upstream components of the Stat3 pathway such as JAK2 and epidermal growth factor receptor (EGFR) (Thilakasiria et al., 2021). In our study, we focused on inhibitors that act by directly inhibiting the SH2 domain of Stat3 proteins in breast cancer cells. Considering their chemical structures, these compounds can be grouped as peptides (SPI), peptidomimetics (CJ-1383, S3I-M2001) and non-peptidic chemical inhibitors (new series of small molecules) (Stattic, STA-21, S3I-201, BP-1- 102). When the results of the studies investigating the effects of these compounds on breast cancer cells with constitutively active Stat3 signaling were examined, it was observed that the compounds showed anticarcinogenic effects such as inhibition of cell viability, migration, induction of apoptosis and inhibition of tumor growth in breast tumor xenograft models. Based on these results, it can be said that the SH2 domain of Stat3 is an important target for breast cancer treatment, worthy of further investigation, and that SH2 domain inhibitors can be used alone or in combination with existing chemotherapeutics, resulting in clinically significant results such as higher efficacy and less toxicity.

References

Alvarez, J. ., Febbo, P. ., Ramaswamy, S., Loda, M., Richardson, A., & Frank, D. . (2005). Identification of a genetic signature of activated signal transducer and activator of transcription 3 in human tumors. *Cancer Research*, *65*(12), 5054–5062.

Banerjee, K., & Resat, H. (2016). Constitutive activation of STAT3 in breast cancer cells: A review. *International Journal of Cancer, 138*(11), 2570–2578.

Berg, T. (2008). Signal Transducers and Activators of Transcription as Targets for Small Organic Molecules. *ChemBioChem*, *9*, 2039–2044.

Buettner, R., Mora, L., & Jove, R. (2002). Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clinical Cancer Research*, *8*(4), 945–954.

Calò, V., Migliavacca, M., Bazan, V., Macaluso, M., Buscemi, M., Gebbia, N., & Russo, A. (2003). STAT proteins: from normal control of cellular events to tumorigenesis. *Journal of Cellular Physiology*, *197*(2), 157–168.

Catlett-Falcone, R., Dalton, W. S., & Jove, R. (1999). STAT proteins as novel targets for cancer therapy. *Current Opinion in Oncology*, *11*(6), 490.

Chen, J., Bai, L., Bernard, D., Nikolovska-Coleska, Z., Gomez, C., Zhang, J., … Wang, H. (2010). Structure-based design of conformationally constrained, cell-permeable STAT3 inhibitors. *ACS Medicinal Chemistry Letters*, *1*(2), 85–89.

Chueh, F. Y., Leong, K. F., & Yu, C. L. (2010). Mitochondrial translocation of signal transducer and activator of transcription 5 (STAT5) in leukemic T cells and cytokine-stimulated cells. *Biochemical and Biophysical Research Communications*, *402*(4), 778–783.

Desrivières, S., Kunz, C., Barash, I., Vafaizadeh, V., Borghouts, C., & Groner, B. (2006). The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. *Journal of Mammary Gland Biology and Neoplasia*, *11*, 75–87.

Furqan, M., Akinleye, A., Mukhi, N., Mittal, V., Chen, Y., & Liu, D. (2013). STAT inhibitors for cancer therapy. *Journal of Hematology & Oncology*, *6*(90).

Gibbs, C. ., Kukekov, V. ., Reith, J. ., Tchigrinova, O., Suslov, O. ., Scott, E. ., … Steindler, D. . (2005). Stem-like cells in bone sarcomas: Implications for tumorigenesis. *Neoplasia*, *7*, 967–976.

Gough, D. ., Corlett, A., Schlessinger, K., Wegrzyn, J., Larner, A. ., & Levy, D. . (2009). Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. *Science*, *324*, 1713–1716.

Groner, B., & von Manstein, V. (2017). Jak Stat signaling and cancer: Opportunities, benefits and side effects of targeted inhibition. *Molecular and Cellular Endocrinology*, *451*, 1–14.

Hao, S., Chen, X., Wang, F., Shao, Q., Liu, J., Zhao, H., … Mao, H. (2018). Breast cancer cell–derived IL-35 promotes tumor progression via induction of IL-35-producing induced regulatory T cells. *Carcinogenesis*, *39*(12), 1488–1496.

Hirsch, H. A., Iliopoulos, D., & Struhl, K. (2013). Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proceedings of the National Academy of Sciences*, *110*(3), 972–977.

Hodge, D. R., Hurt, E. M., & Farrar, W. L. (2005). The role of IL-6 and STAT3 in inflammation and cancer. *European Journal of Cancer*, *41*, 2502–2512.

Hughes, K., & Watson, C. J. (2018). The multifaceted role of STAT3 in mammary gland involution and breast cancer. *International Journal of Molecular Sciences*, *19*(6), 1695.

Ibarra-Sanchez, M., Simoncic, P., Nestel, F., Duplay, P., Lapp, W., & Tremblay, M. (2000). The T-cell protein tyrosine phosphatase. *Seminars in Immunology*, *12*(4), 379–386.

Igelmann, S., Neubauer, H. A., & Ferbeyre, G. (2019). STAT3 and STAT5 Activation in Solid Cancers. *Cancers*, *11*(1428).

Jr, J. E. D., Kerr, I. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional ac- tivation in response to IFNs and other extracellular signaling proteins. *Science*, *264*(5164), 1415–1421.

Kamran, M. Z., Patil, P., & Gude, R. P. (2013). Role of STAT3 in cancer metastasis and translational advances. *BioMed Research International*.

Kim, S. Y., Kang, J. W., Song, X., Kim, B. K., Yoo, Y. D., Kwon, Y. T., & Lee, Y. J. (2013). Role of the IL-6-JAK1-STAT3-Oct-4 pathway in the conversion of non-stem cancer cells into cancer stem-like cells. *Cellular Signalling*, *25*(4), 961–969.

Kim, T. K., & Maniatis, T. (1996). Regulation of interferon-γ-activated STAT1 by the ubiquitin-proteasome pathway. *Science*, *273*(5282), 1717–1719.

Kortylewski, M., Jove, R., & Yu, H. (2005). Targeting STAT3 affects melanoma on multiple fronts. *Cancer and Metastasis Reviews*, *24*, 315–327.

Krebs, D., & Hilton, D. (2001). SOCS proteins: Negative regulators of cytokine signaling. *Stem Cells*, *19*, 378–387.

Kritikou, E. ., Sharkey, A., Abell, K., Came, P. ., Anderson, E., Clarkson, R. ., & Watson, C. . (2003). A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. *Development*, *130*, 3459–3468.

Li, M., Liu, X., Robinson, G., Bar-Peled, U., Wagner, K. ., Young, W. ., … Furth, P. A. (1997). Mammary-derived signals activate programmed cell death during the first stage of mammary gland involution. *Proceedings of the National Academy of Sciences*, *94*(7), 3425–3430.

Li, Z., Chen, Y., An, T., Liu, P., Zhu, J., Yang, H., … Yang, X. (2019). Nuciferine inhibits the progression of glioblastoma by suppressing the SOX2-AKT/STAT3-Slug signaling pathway. *Journal of Experimental & Clinical Cancer Research*, *38*(1), 1–15.

Lieblein, J. ., Ball, S., Hutzen, B., Sasser, A. ., Lin, H. ., Huang, T. ., … Lin, J. (2008). STAT3 can be activated through paracrine signaling in breast epithelial cells. *BMC Cancer*, *8*, 1–14.

Lim, C. P., & Cao, X. (2006). Structure, function, and regulation of STAT proteins. *Molecular BioSystems*, *2*, 536–550.

Liu, B., & Shuai, K. (2003). The PIAS protein family and Tc-PTP. In P. B. Sehgal, D. E. Levy, & T. Hirano (Eds.), *Signal Transducers and Activators of Transcription (STATs): Activation and Biology* (pp. 75–85). Kluwer Academic Publishers.

Lo, H. ., Hsu, S. ., Xia, W., Cao, X., Shih, J. ., Wei, Y., … Hung, M. . (2007). Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. *Cancer Research*, *67*, 9066–9076.

Logotheti, S., & Pützer, B. M. (2019). STAT3 and STAT5 Targeting for Simultaneous Management of Melanoma and Autoimmune Diseases. *Cancers*, *11*(1448).

Ma, J., Qin, L., & Li, X. (2020). Role of STAT3 signaling pathway in breast cancer. *Cell Communication and Signaling*, *18*(33), 1–13.

Macias, E., Rao, D., Carbajal, S., Kiguchi, K., & DiGiovanni, J. (2014). Stat3 binds to mtDNA and regulates mitochondrial gene expression in keratinocytes. *Journal of Investigative Dermatology*, *134*(7), 1971–1980.

Marotta, L. ., Almendro, V., Marusyk, A., Shipitsin, M., Schemme, J., Walker, S. ., … Lee, H, K. (2011). The JAK2/STAT3 signaling pathway is required for growth of CD44 + CD24- stem cell-like breast cancer cells in human tumors. *The Journal of Clinical Investigation*, *121*(7), 2723–2735.

Marotta, L. L. C., Almendro, V., Marusyk, A., Shipitsin, M., Schemme, J., Walker, S. R., … Kornelia Polyak. (2011). The JAK2/STAT3 signaling pathway is required for growth of CD44 + CD24- stem cell-like breast cancer cells in human tumors. *The Journal of Clinical Investigation*, *121*(7), 2723–2735.

McMurray, J. S. (2006). A New Small-Molecule Stat3 Inhibitor. *Chemistry & Biology*, *13*(11), 1123–1124.

Moriggl, R., Sexl, V., Kenner, L., Duntsch, C., Stangl, K., Gingras, S., … Beug, H. (2005). Stat5 tetramer formation is associated with leukemogenesis. *Cancer Cell*, *7*(1), 87–99.

Park, H. ., Li, J., Hannah, R., Biddie, S., Leal-Cervantes, A. ., Kirschner, K., … Green, A. . (2016). Cytokine-induced megakaryocytic differentiation is regulated by genome-wide loss of a uSTAT transcriptional program. *The EMBO Journal*, *35*(6), 580–594.

Sadowski, H. B., Shuai, K., Jr, J. E. D., & Gilman, M. Z. (1993). A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science*, *261*(5129), 1739–1744.

Sala, D., Cunningham, T. J., Stec, M. J., Etxaniz, U., Nicoletti, C., Dall'Agnese, A., … Sacco, A. (2019). The Stat3-Fam3a axis promotes muscle stem cell myogenic lineage progression by inducing mitochondrial respiration. *Nature Communications*, *10*(1), 1796.

Schust, J., Sperl, B., Hollis, A., Mayer, T. U., & Berg, T. (2006). Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chemistry & Biology*, *13*(11), 1235–1242.

Siddiquee, K. A. Z., Gunning, P. T., Glenn, M., Katt, W. P., Zhang, S., Schroeck, C., … Turkson, J. (2007). An oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing and induces antitumor cell effects. *ACS Chemical Biology*, *2*(12), 787–798.

Siddiquee, K., Zhang, S., Guida, W. C., Blaskovich, M. A., Greedy, B., Lawrence, H. R., … Turkson, J. (2007). Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proceedings of the National Academy of Sciences*, *104*(18), 7391–7396.

Simononic, P., Lee-Loy, A., Barder, D., Tremblay, M., & McGlade, C. (2002). The T cell protein tyrosine phosphatase is a negative regulator of Janus family kinases 1 and 3. *Current Biology*, *12*, 446–453.

Song, H., Wang, R., Wang, S., & Lin, J. (2005). A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. *Proceedings of the National Academy of Sciences*, *102*(13), 4700–4705.

Stein, T., Salomonis, N., & Gusterson, B. A. (2007). Mammary Gland Involution as a Multi-step Process. *Journal of Mammary Gland Biology and Neoplasia*, *12*, 25–35.

Sullivan, N. ., Sasser, A. ., Axel, A. ., Vesuna, F., Raman, V., Ramirez, N., … Hall, B. . (2009). Interleukin-6 induces an epithelial–mesenchymal transition phenotype in human breast cancer cells. *Oncogene*, *28*, 2940–2947.

Takeda, K., Noguchi, K., Shi, W., Tanaka, T., Matsumoto, M., Yoshida, N., … Akira, S. (1997). Targeted disruption of the mouse Stat3 gene leads to

early embryonic lethality. *Proceedings of the National Academy of Sciences*, *94*(8), 3801–3804.

Tawara, K., Scott, H., Emathinger, J., Ide, A., Fox, R., Greiner, D., … Jorcyk, C. (2019). Co-expression of VEGF and IL-6 family cytokines is associated with decreased survival in HER2 negative breast cancer patients: subtype-specific IL-6 family cytokine-mediated VEGF secretion. *Translational Oncology*, *12*(2), 245–255.

Tawara, K., Scott, H., Emathinger, J., Wolf, C., LaJoie, D., Hedeen, D., … Jorcyk, C. (2019). HIGH expression of OSM and IL-6 are associated with decreased breast cancer survival: synergistic induction of IL-6 secretion by OSM and IL-1beta. *Oncotarget*, *10*, 2068–2085.

Thilakasiria, P. S., Dmelloa, R. S., Nerob, T. L., Parkerb, M. W., Ernsta, M., & Chand, A. L. (2021). Repurposing of drugs as STAT3 inhibitors for cancer therapy. *Seminars in Cancer Biology*, *68*, 31–46.

Tolomeo, M., & Cascio, A. (2021). The Multifaced Role of STAT3 in Cancer and Its Implication for Anticancer Therapy. *International Journal of Molecular Sciences*, *22*(2), 603.

Turkson, J., & Jove, R. (2000). STAT proteins: novel molecular targets for cancer drug discovery. *Oncogene*, *19*(2000), 6613–6626.

Tzeng, Y.-D. T., Liu, P.-F., Li, J.-Y., Liu, L.-F., Kuo, S.-Y., Hsieh, C.-W., … Shu, C.-W. (2018). Kinome-wide siRNA screening identifies Src-enhanced resistance of chemotherapeutic drugs in triple-negative breast cancer cells. *Frontiers in Pharmacology*, *9*, 1285.

Valeta-Magara, A., Gadi, A., Volta, V., Walters, B., Arju, R., Giashuddin, S., … J, R. S. (2019). No Inflammatory Breast Cancer Promotes Development of M2 Tumor-Associated Macrophages and Cancer Mesenchymal Cells through a Complex Chemokine Network. *Cancer Research*, *79*(13), 3360–3371.

Verhoeven, Y., Tilborghs, S., Jacobs, J., Waele, J. De, Quatannens, D., Deben, C., … Dam, P. A. van. (2020). The potential and controversy of targeting STAT family members in cancer. *Seminars in Cancer Biology*, *60*(2020), 41–56.

Walker, S.R, Nelson, E. ., Zou, L., Chaudhury, M., Signoretti, S., Richardson, A., & Frank, D. . (2009). Reciprocal effects of STAT5 and STAT3 in breast cancer. *Molecular Cancer Research*, *7*(6), 966–976.

Walker, Sarah R., Xiang, M., & Frank, D. A. (2014). Distinct roles of STAT3 and STAT5 in the pathogenesis and targeted therapy of breast cancer. *Molecular and Cellular Endocrinology*, *382*(1), 616–621.

Wang, T., Fahrmann, J. F. H. L., Li, Y.-J., Tripathi, S. C., Yue, C., Zhang, C., … Yu, H. (2018). JAK/STAT3-regulated fatty acid β-oxidation is critical for

breast cancer stem cell self-renewal and chemoresistance. *Cell Metabolism*, *27*(1), 136–150.

Weaver, A., & Silva, C. (2007). Signal transducer and activator of transcription 5b: a new target of breast tumor kinase/protein tyrosine kinase 6. *Breast Cancer Research*, *9*(6), R79.

Wegrzyn, J., Potla, R., Chwae, Y.-J., Sepurı, N. B. V., Zhang, Q., Koeck, T., … Larner, A. C. (2009). Function of mitochondrial Stat3 in cellular respiration. *Science*, *323*, 793–797.

Xin, P., Xua, X., Denga, C., Liua, S., Wang, Y., Zhou, X., … Sun, S. (2020). The role of JAK/STAT signaling pathway and its inhibitors in diseases. *International Immunopharmacology*, *80*(2020), 106210.

Xiong, A., Yang, Z., Shen, Y., Zhou, J., & Shen, Q. (2014). Transcription Factor STAT3 as a Novel Molecular Target for Cancer Prevention. *Cancers*, *6*, 926–957.

Yadav, A., Kumar, B., Datta, J., Teknos, T. ., & Kumar, P. (2011). IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. *Molecular Cancer Research*, *9*, 1658–1667.

Yue, P., & Turkson, J. (2009). Targeting STAT3 in cancer: how successful are we? *Expert Opinion on Investigational Drugs*, *18*(1), 45–56.

Zhang, Q., Raje, V., Yakovlev, V. A., Yacoub, A., Szczepanek, K., Meier, J., … Larner, A. C. (2013). Mitochondrial localized Stat3 promotes breast cancer growth via phosphorylation of serine 727. *Journal of Biological Chemistry*, *288*(43), 31280–31288.

Zhang, X., Yue, P., Page, B. D. G., Li, T., Zhao, W., Namanja, A. T., … Turkson, J. (2012). Orally bioavailable small-molecule inhibitor of transcription factor Stat3 regresses human breast and lung cancer xenografts. *Proceedings of the National Academy of Sciences*, *109*(24), 9623–9628.

Zhao, W., Jaganathan, S., & Turkson, J. (2010). A cell-permeable Stat3 SH2 domain mimetic inhibits Stat3 activation and induces antitumor cell effects in vitro. *Journal of Biological Chemistry, 285*(46), 35855–35865.

Zhao, Y., Zeng, C., Tarasova, N. ., Chasovskikh, S., Dritschilo, A., & Timofeeva, O. . (2013). A new role for STAT3 as a regulator of chromatin topology. *Transcription*, *4*, 227–231.