

## Review Article



# The Role of STAT1 in T Helper Cell Differentiation during Breast Cancer Progression

Sayantana Banik , Sudeshna Rakshit , Koustav Sarkar

Department of Biotechnology, SRM Institute of Science and Technology, Kattankulathur, India

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### Correspondence to

Koustav Sarkar

Department of Biotechnology, SRM Institute of Science and Technology, Potheri, SRM Nagar, Kattankulathur 603203, India.

E-mail: koustavsarkar@gmail.com

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### ORCID iDs

Sayantana Banik

<https://orcid.org/0000-0002-8070-6390>

Sudeshna Rakshit

<https://orcid.org/0000-0002-7496-3155>

Koustav Sarkar

<https://orcid.org/0000-0002-0696-6688>

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## ABSTRACT

Members of the signal transducer and activator of transcription (STAT) protein family are intracellular transcription factors that facilitate several facets of cellular immunity, proliferation, apoptosis, and differentiation. They are principally stimulated by membrane receptor-associated Janus kinases. Dysregulation of this pathway is often detected in primary tumors and hints at augmented angiogenesis, which enriches tumor persistence and immunosuppression. STAT proteins play indispensable roles in cytokine signaling and T helper (Th) cell differentiation. Among STAT proteins, STAT1 plays a vital role in interferon signaling, which initiates the expression of genes encoding proteins with antitumor and apoptotic roles. STAT1 signaling is essential for Th1 cell differentiation. Several studies have also shown the role of STAT1 as a tumor suppressor in breast cancer, which is the most common intrusive malignancy and the second most common cause of cancer death in women. Herein, we review the intricate STAT1-mediated molecular mechanisms associated with Th cell differentiation and anti-tumor function in breast cancer.

**Keywords:** Breast neoplasms; Interferon; STAT; Th cell

## INTRODUCTION

The signal transducer and activator of transcription (STAT) family was first discovered in the early 1990s due to its unique features. The last 15 years have seen extraordinary advances in the knowledge of STAT structure, function, and regulation. As the name suggests, STATs activate gene transcription by transducing signals from the cell membrane to the nucleus, thereby bypassing the participation of secondary messengers. The first STAT genes to be identified in the interferon (IFN) signal transduction pathways in mammalian cells were *STAT1* and *STAT2* [1]. *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5* (*STAT5A* and *STAT5B*), and *STAT6* are members of the mammalian STAT family. *STAT3* and *STAT5*, in particular, have been linked to cancer progression, while *STAT1* suppresses tumor development. *STAT1* has been shown to play an anti-tumor role by regulating the immune system and promoting tumor immune surveillance. Although some cytokines and growth factors can activate multiple STAT proteins, some STATs are activated with a high level of specificity; hence, multiple ligands may activate individual STAT proteins but certain cytokines preferentially activate specific STATs. For instance, IFN- $\gamma$  preferentially activates *STAT1* [2]. The physiological role

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of individual STAT proteins has been investigated using “knockout” mice with a null allele for the genes in question. The transcription factors STAT4 and STAT6 are required for T helper 1 (Th1) cell and Th2 responses, respectively. Th cells are essential components of the acquired immune system and are responsible for a variety of acquired immune reactions. Th cells stimulate B cell activation to release immunoglobulins and digest ingested microorganisms and initiate the destruction of tumor cells. The Janus kinase (JAK)-STAT signaling pathway plays a critical role in transducing signals from various cytokines to achieve distinct transcriptional outcomes. In T cells, this pathway has been well studied in terms of the regulation of T cell differentiation. Among the 7 mammalian STAT family members, STAT1 is important for the induction of Th1 cells downstream of IFN- $\gamma$  following the induction of the T-bet transcription factor. STAT1 has also been shown to suppress regulatory T cell (Treg differentiation). These proinflammatory properties of STAT1 are important for controlling infections; patients with loss-of-function mutations in STAT1 are susceptible to viral/mycobacterial infections [3]. While T cell infiltration is frequently observed in breast cancer, the immunological course of tumor progression in breast cancer is specifically regulated by Th cells. STAT1 modulates Th cell polarization. Transcription factors responsible for the suppression of cytokine signaling and protein inhibitors can activate STAT1 and tyrosine phosphatases, which determine the period and end of the signaling. Disruption of this pathway in Th cells can lead to different issues, including malignant growth [4]. This review describes the role of STAT1 in Th cell differentiation during breast cancer progression.

## MOLECULAR STRUCTURE AND FUNCTIONAL DOMAINS OF STAT1

The crystal structure of unphosphorylated STAT1 was reported in 2005. This structure includes a 5-residue phosphopeptide from the  $\alpha$ -chain of the IFN- $\gamma$  receptor and a tetrameric configuration. The 2 dimer interfaces—one between 2 N-domains and another between 2 core fragments—comprise the coiled-coil domain to the Tyr-phosphorylated tail portion [5]. STAT proteins contain 700–850 amino acids and share many functional domains. The 6 STAT1 domains are the 1) N-domain/STAT protein interaction domain, 2) coiled-coil domain/STAT all-alpha domain, 3) DNA binding domain, 4) linker domain, 5) Src homology 2 (SH2) domain, and 6) transactivation domain (TAD). A Tyr residue at the C-terminus of all STAT family members is phosphorylated when activated. It can interact with the SH2 domain of the dimer to form intermolecular interactions.  $\alpha$  isoforms refers to the entire STAT family, while shorter isoforms are referred to as  $\beta$ ,  $\gamma$ , or  $\delta$ . The isoforms lack the phospho-Ser residue but retain the critical invariant phospho-Tyr residue due to carboxyl truncations. Similar to  $\alpha$  isoforms, the  $\beta$  isoforms are efficiently Tyr phosphorylated, form homo- or heterodimers with isoforms, and bind DNA. However, the overexpression of STAT1 [6]  $\beta$  isoforms may act as a dominant-negative factor

The activities of various STAT domains have been extensively studied. The N-domain is involved in dimerization and tetramerization [7], as well as protein-protein interactions, while the coiled-coil domain communicates with other proteins, such as interferon regulatory factor (IRF)-9/p48, for STAT signaling [8]. In addition to the domains that participate in nuclear import and export, the N-domain, K410/K413, or L407 in the DNA-binding domain of STAT1 also interacts with importin  $\alpha$ 5/NPI-1 during cytokine-induced nuclear import [9]. When activated, STATs are rapidly phosphorylated in the cytoplasm and translocated to the nucleus. STATs also act as transcription factors by binding specific DNA

sequences to activate gene transcription [6]. This is the primary function of STAT proteins. STAT-binding elements are found in 2 types of response elements. The first is the IFN-sensitive response element, which has a consensus sequence of AGTTTN3TTTC and is bound by the multimeric complex IFN-stimulated gene factor 3 (ISGF3), which includes STAT1a, STAT1b, STAT2, and p48/IRF-9 and is only involved in IFN signaling. ISGF3 targets the *ISG15*, *ISG54*, *6-16*, and *2-5A* synthetase genes. The second response element is the gamma-activated sequence (GAS); GAS was first reported in IFN signaling, has the sequence TTCN2-4GAA, and is bound by STAT homo/heterodimers. Contrary to popular opinion, phospho-STAT may function as a transcriptional co-regulator in the presence of other transcription factors. The constitutive activation of some STATs or the upregulation of phospho-STAT activity has been linked to cancers, including breast cancer [10].

## PHYSIOLOGICAL FUNCTION AND MOLECULAR ACTIVITIES OF STAT1

In general, STAT1 is considered a tumor suppressor for its ability to regulate the immune system and stimulate tumor immune surveillance. However, the cell-autonomous roles of STAT1 in tumor formation are poorly understood. Recent findings have demonstrated that STAT1 suppresses mammary gland (mouse) tumorigenesis via immune regulatory and tumor cell-specific functions [11]. STAT1 functions as a sequence-specific DNA-binding factor and is involved in cellular responses to cytokines. All members of the STAT family are involved in cytokine and growth factor receptor signaling. STAT1 is the most important STAT protein that is activated by IFN- $\gamma$ . Many of the effects of IFN are mediated by the direct activation by STAT1 of immune effector genes. STAT1 predominantly represses tumor function; however, not all STAT protein family members participate in tumor formation in humans [12]. Additionally, STAT1 is a key factor in the natural immune response. The IFN/STAT1 signaling pathway facilitates crosstalk between tumor cells and their host microenvironment [13]. STAT1 is a DNA-binding protein that functions downstream of the IFN I and II receptors [14]. Phosphorylation of tyrosine 701 and serine 727 in the TAD controls STAT1 function. The transition of STAT1 between different dimer conformations is regulated by the phosphorylation of Y701, which activates a parallel dimer conformation mediated by phosphotyrosine; in contrast, unphosphorylated STAT1 can dimerize in an antiparallel conformation: SH2 domain interactions which mediate a specific association between a STAT and the cytoplasmic domain of the appropriate receptor, permitting increased DNA binding and nuclear retention of the protein. The phosphorylation of STAT1 and tyrosine is initiated by JAKs activated by IFNs and cytokine reactions. However, intrinsic tyrosine kinase activity with receptor-like epidermal growth factor receptor (EGFR) can initiate STAT1 phosphorylation at the tyrosine residue Y701. The receptor-associated JAKs that are activated in response to IFNs and other cytokines phosphorylate STAT1 and Y701. STAT1 phosphorylation at Y701 can also be mediated by receptors with intrinsic tyrosine kinase activity, such as EGFR. The phosphorylation of STAT1 at S727 is required for gene transactivation in response to IFNs. Acetylation of STAT1 at lysine (K) 410 and K413 inhibits its function by preventing DNA binding and encouraging an anti-parallel conformation of STAT1 dimers, which facilitates Y701 dephosphorylation. The SH2 domains bind to tyrosine-phosphorylated proteins and mediate the formation of signaling complexes. A phosphotyrosine binding domain has also been discovered, which binds to tyrosine-phosphorylated targets but differs structurally from SH2 domains [15].

## STAT1 ACTIVATION

A variety of cytokines, including IFNs and interleukins (ILs), as well as certain growth factors and hormones, activate STAT proteins. Many different, and sometimes overlapping ligands, activate STAT1, STAT3, STAT5A, and STAT5B. IFN-A/B and IFN-C both induce STAT1 expression. Antiviral and antibacterial activities, apoptosis, and tumor suppression are all influenced by these proteins. STATs can be activated in 5 different ways [16]. In cytokine signaling, the first is the well-known classical JAK-STAT pathway. Cytokine binding causes receptor dimerization, followed by the activation of receptor-associated tyrosine kinases (JAKs), which transphosphorylate the receptor's intracellular domain, allowing latent cytoplasmic STATs to dock at these phospho-Tyr residues [17]. The SH2 domain attracts STATs to the receptor, and JAKs phosphorylate STATs on a particular Tyr residue on their cytoplasmic tails. STATs are homo- or heterodimerized by reciprocal binding with the SH2 domain of the partner dimer. These STAT dimers are delivered from the receptor and translocated into the nucleus through importins, which directly bind to specific DNA binding elements and initiate cytokine-responsive gene transcription. In human breast cancer cell lines, receptors with unique intrinsic tyrosine kinase activities, such as the epidermal growth factor (EGF), platelet-derived growth factor, and fibroblast growth factor receptors, activate STATs directly or indirectly through JAKs involved in EGF signaling [18]. Some viral oncoproteins, such as v-src, v-Fps, v-Sis, polyomavirus middle T antigen, and v-abl, activate STAT through non-receptor tyrosine kinases [19]. G-protein coupled receptors, including chemokine receptors like MIP-1 and RANTES, activate STAT1 and 3 in T cells when they bind to chemokines [20]. STAT activation can also be mediated by specific proteins that act as adaptors, bringing JAKs closer together for STAT activation. STAT activation occurs when there is a high concentration of phosphorylated STAT1 in the nucleus, which is immediately inactivated when the half-life of phosphorylated STAT1 is increased. The nuclear export of STATs is a cytokine-induced chromosome region maintenance 1/exportin1-dependent process and involves binding to importin [21].

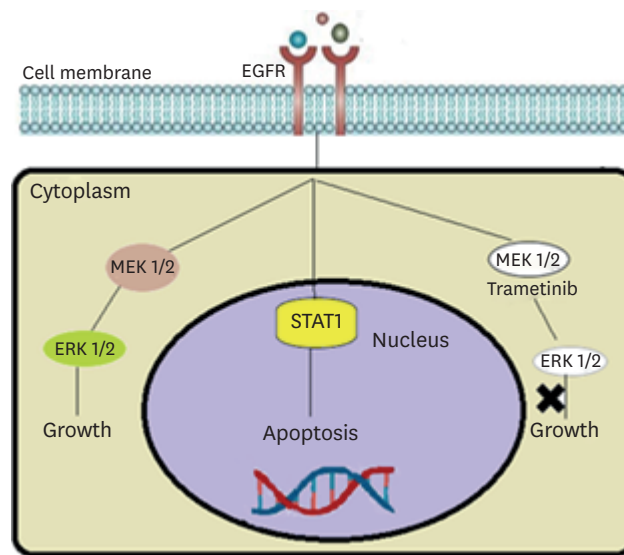
## ROLE OF STAT1 AS A TUMOR SUPPRESSOR

STAT1 is required for IFN signaling [22], which is a potent growth inhibitor and apoptosis promoters, in addition to being required for innate immunity. Stat1-deficient mice do not develop spontaneous tumors that are highly susceptible to chemical carcinogens [23]. Crossing the Stat1 mutation into a p53-deficient context results in animals in which tumors are more easily converted and with a wider range of tumor forms than p53 single mutants. Tumors derived from carcinogen-treated animals transplanted into Stat1-deficient animals develop more quickly, suggesting that Stat1 is needed for tumor surveillance in the host [24]. Stat1's ability to upregulate caspases and the cyclin-dependent kinase inhibitor p21 explain why it is needed for apoptosis and growth arrest in some cell types [25]. The upregulation of p21 by STAT1 in mammary cells tends to include BRCA1, which is often lost in familial and other forms of breast cancer [25]. The p53 gene is a tumor suppressor and an effective cell growth inhibitor [26]. In cancer, the p53 protein stops cell cycle progression in a variety of ways and initiates cell death [27]. STAT1 and p53 can activate the p21 gene promoter [28] and bind to the p300/CREB binding protein at different sites to form a complex to regulate the p53-dependent apoptotic signaling pathway [29]; however, *STAT1* knockout in p53-deficient animals caused uncontrollable tumor growth and a broad variety of tumors [23]. STAT1 inhibited Mdm2, which facilitates the ubiquitination-mediated degradation of p53 protein [30]. STAT1 and p53 work

together to prevent tumor growth by inducing apoptosis. The cytotoxic activity of p53 inducers such as fludarabine is related to increased STAT1 transcriptional activation. These agents can activate p53, which may lead to the collateral activation of STAT1.

## MECHANISM OF STAT1 IN PROSURVIVAL SIGNALING

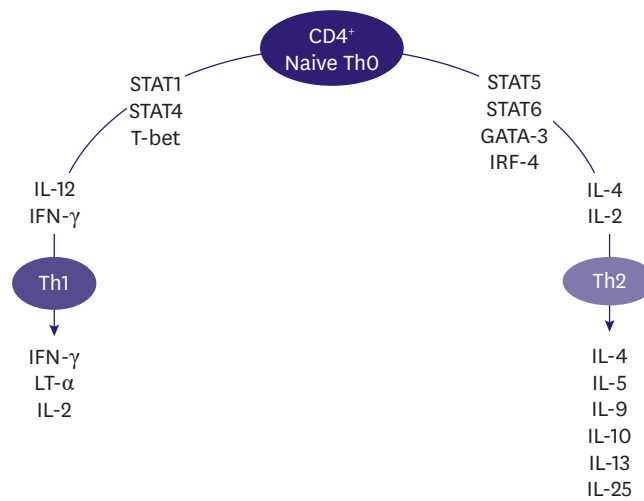
Many cancers, including breast cancer, demonstrate EGFR overexpression. By inhibiting STAT1 activation while facilitating STAT3 activation, EGFR aids in the removal of malignant cells [31]. STAT transcription factors control many normal and pathological processes, including proliferation, apoptosis, and inflammation, as well as tumorigenesis and tumor progression. The nuclear activity of EGFR is primarily responsible for EGFR control of STAT1 expression. STAT3 is an oncogenic transcription factor that binds to the STAT1 promoter and works in concert with nuclear EGFR to improve STAT1 gene expression. Structural characterization of the promoter revealed the existence of 4 functional STAT3-binding sites in the human STAT1 gene promoter as well as their significance in STAT1 co-regulation by EGFR. Recent research suggests that constitutive EGFR signaling induced by receptor mutations is distinct and mutually exclusive from ligand-induced signaling. When activated by one of its ligands, EGFR causes cell differentiation and proliferation. The receptor is found on the cell surface, where ligand binding activates a tyrosine kinase in the intracellular region of the receptor. This tyrosine kinase phosphorylates several intracellular substrates, which activate pathways leading to cell development, DNA synthesis, and oncogene expression [32]; ERK1 and ERK2 are serine/threonine protein kinases that are involved in the MEK/ERK signaling cascade. Cell adhesion, cell cycle development, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription are only a few of the processes controlled by this cascade [33]. Directly targeting WT-EGFR with a nuclear-localized gefitinib conjugate prevented STAT1 activation, resulting in increased cell growth, since this compound does not impair ERK1/2 signaling by EGFR. Furthermore, unconjugated gefitinib inhibited STAT1-mediated apoptotic signaling downstream of ligand-activated WT-EGFR at a much lower concentration than that required to inhibit ERK1/2-mediated proliferative signaling. Pro-growth and pro-survival signaling pathways, such as Erk1/2, dominate EGFR signaling in primary breast cancer. STAT1 signaling is increased in metastatic breast cancer, which supports EGF-induced apoptosis. Pharmacologic inhibition of MEK/ERK signaling with the allosteric inhibitor trametinib will bias EGFR signaling toward STAT1-mediated apoptosis [34], as shown in **Figure 1**. However, the effect of STAT1 inhibition on the growth of ER<sup>+</sup> epithelial (mammary) cells is affected by type I IFN. IFN-induced STAT1 signaling primarily activates genes that mediate immune functions, antiviral and/or ant pathogen functions, cell proliferation suppression, and apoptosis induction [35]. Thus, STAT1 signaling is widely regarded as a cancer-suppressive pathway and also essential for tumor immune surveillance. STAT1 upregulation was shown to induce the activation of subsets of ISGs that are associated with increased tumor cell resistance to genotoxic stress and/or tumor development, contrary to the previously benign view of STAT1 signaling [36]. Preclinical studies showed that the IFN/STAT1 pathway mediates radioresistance in the tumor microenvironment (TME) by shielding immune responses and triggering survival signaling pathways. Transcriptionally activated STAT1 is needed to inhibit ER<sup>+</sup> tumor growth in mice. In mammary tissue, type I IFN is a key factor for its downregulation in STAT1-deficient tumor cells. IRF1 functions as a downstream regulator of STAT1 and is required for insufficient STAT1-IRF1 status in tumor progression. Till now this is not proved that the cause of downregulation of IFN type I in Breast cancer is related to a lower level of STAT1 [37].



**Figure 1.** Schematic representation of EGFR signaling related to STAT1-facilitated apoptosis through the pharmacologic inhibition of MEK/ERK signaling. EGFR = epidermal growth factor receptor; STAT = signal transducer and activator of transcription.

## ROLE OF STAT1 IN T CELL DIFFERENTIATION

IFN- $\gamma$  is a central cytokine in the tumor-suppressive network that works by interacting with type II IFN receptors and IFN signals, primarily through the JAK-STAT intracellular signal transduction pathway, to activate IFN-inducible genes [35]. IFN activates the JAK/STAT1 signaling pathway by binding to a receptor. STAT1 is well-known for its role in the induction of Th1 cells downstream of IFN by inducing the transcription factor T-bet. IFN suppresses Th2 cell differentiation and simultaneous IL-4 synthesis [38], as shown in **Figure 2**. IL-4 is a cytokine that induces the differentiation of naive Th cells (Th0 cells) to Th2 cells [39]. Th1 cells cannot induce IL-4 expression. Th0 cells can be divided into Th2-inducing conditions. IFN-J signaling acts as a key factor in suppressing IL-4 in Th1 cells to stabilize the Th1 phenotype. Furthermore, this has been shown to activate IL-4 expression by impeding STAT6 phosphorylation. In Th1 cells, STAT1, which transduces IFN-J signaling, interferes with the repression of IL-4 expression [40]. A combination of STAT1 and CD4<sup>+</sup> T cells may decrease IFN-J and increase IL-4 levels. Under unpolarized conditions, these cells can transform into Th2 cells. Then, Th1 cells and STAT1 decrease IFN-J and T-bet levels. However, these cannot repress STAT6 signaling and IL-4 and GATA-3 expression. Under Th2-inducing conditions, STAT1 and Th1 cells can differentiate into IL-4 cells. Surprisingly, the reaction of T-bet with STAT1 and Th1 suppresses the action and restores IFN-J function, suggesting that STAT1 can inhibit the conversion of IL-4 into T-bet. T-bet is found in a variety of innate and adaptive immune cell forms, including myeloid and lymphoid lineages. T-bet controls IFN expression by recruiting chromatin-modifying enzymes, which encourage permissive chromatin marks at Th1 cell-specific loci. IFN- $\gamma$  signaling has been shown to stabilize Th1 phenotype in part through repressing STAT6 signaling. It can act in STAT1-dependent or -independent manners. STAT1-T-bet positive regulatory loop plays a critical role in repressing IL4 gene in Th1 cells. It promotes IFN- $\gamma$  production as well as represses IL-4 expression by inhibiting STAT6 signaling, antagonizing GATA3, and repressing epigenetic modifications and chromosomal remodeling [41]. The function of STAT1 in tumor progression and immune cell infiltration is a primary explanation for the research interest in this topic [42]. The tumor



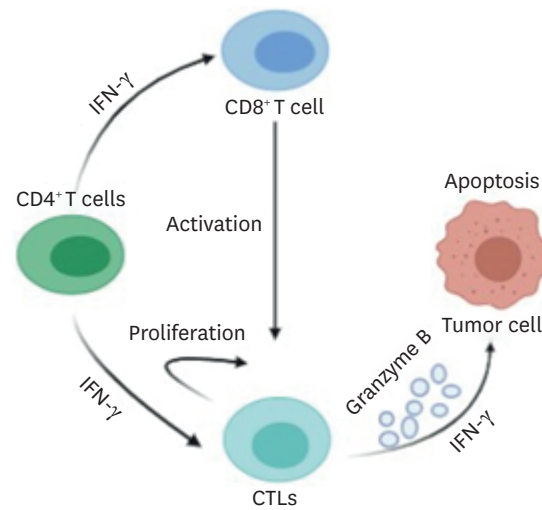
**Figure 2.** Involvement of signatory cytokines and corresponding transcription factors in Th1 and Th2 differentiation.

Th = T helper; IFN = interferon; LT = lymphotoxin; IL = interleukin; STAT = signal transducer and activator of transcription.

suppressor role of STAT-1 has been mainly attributed to its capacity to promote apoptosis and to inhibit proliferation in experimental models [13].

## EFFECTS OF TH CELLS DURING BREAST CANCER PROGRESSION

T lymphocytes play 3 types of roles in malignancy, especially breast cancer. The priming of CD4<sup>+</sup> and CD8<sup>+</sup> cells specific for tumor-associated antigens, for example, is required for effective systemic antitumor immunity. Th cells may help to prime major histocompatibility complex (MHC) class I-limited CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) by providing regulatory signals. Activated CTLs primarily serve as immune effectors that cause tumor cell apoptosis. As shown in **Figure 3**, tumor immunity is normally mediated by CTLs, whose activation and stimulation are aided by Th1 cytokines [43]. CTLs are also a group of immune cells that directly kill other cells. They are essential in host protection against infection by virus and other intracellular pathogens that replicate in the cytoplasm of host cells [44]. The entire CTL activation process alone is lacking to intervene in an anticancer reaction. Individually, CD8<sup>+</sup> cells cannot function the actuation of CD4<sup>+</sup> cells. Recent studies have clarified the involvement of Th cell response in the immune response [45]. Th cells act as a key factor in advancing the immune response by activating antigen-specific effectors to protect against malignancies and infection and help innate immune system cells such as mast cells, eosinophils, and macrophages. Studies have also demonstrated the roles of Th cells in tumor immunity and against foreign pathogens. CD4<sup>+</sup> T cells mainly mediate anti-tumor immunity by assisting CD8<sup>+</sup> CTL and antibody responses, secreting effector cytokines such as IFN and tumor necrosis factor, and providing direct cytotoxicity against tumor cells [46]. CTLs with CD8 are the most effective immune cells for fighting cancer. CTLs become depleted with cancer progression due to immune-related tolerance and immunosuppression in the TME, both of which favor adaptive immune resistance. CD8<sup>+</sup> cells are associated with the lysis of tumor cells, which are specific antigen tumor cells. Th1 cells can upgrade cytotoxic CD8<sup>+</sup> T cell movement and can likewise initiate antigen-introducing dendritic cells (DCs) to kill the tumor.



**Figure 3.** CD4<sup>+</sup> T cell-mediated interferon- $\gamma$  signaling regulates CD8<sup>+</sup> CTL activation for tumor cell apoptosis. CTL = cytotoxic T lymphocyte; IFN = interferon.

In contrast, Th2 cells repress DC function and suppress CD8<sup>+</sup> cell action. Finally, suppressing CD4<sup>+</sup> FOXP3<sup>+</sup> T cells induce CTL function by CD8<sup>+</sup> cells, helping to kill the tumors.

## TH CELL ACTIVATION

Th cells are activated in a multistep process that starts with antigen-presenting cells (APCs) such as macrophages or DCs. Th cells are activated in one of 2 ways: by cytokine stimulation or by a costimulatory reaction between B7, a signaling protein found on the surface of APCs, and CD28, a receptor protein found on the surface of Th cells [42]. CTLs have been identified as tumor-killing cells in a variety of cancers. These cells have T cell receptors that recognize cancer-specific antigens. These cells undergo clonal expansion after recognizing the antigen and before circulating throughout the body in search of the antigenic source cell population. Several studies have attempted to characterize the role of CTLs in the development of breast cancer. These cell infiltrates are linked to a higher histological grade and basal phenotype, as well as estrogen receptor and progesterone receptor expression [47]. CTL immunity in tumor-specific immune responses is regulated by Th cells. Th cell depletion reduces the ability to reject human leukocyte antigen (HLA)-class I-negative tumors and the specific targeting of CTL. Th1 and Th2 play crucial roles in the anti-tumor response. Moreover, animal experiments showed that the removal of Th cells reduced the response capacity against the tumor. When the immune response is low, the macrophage tumor-killing process is activated [48].

## TH1/TH2 BALANCE IN BREAST CANCER

Th1/Th2 imbalances can lead to various illnesses, as well as their complications. Patients with advanced cancer often have impaired cell-mediated immunity as a result of a Th1/Th2 switch [49]. Switching from one cytokine pattern to another, on the other hand, may be extremely useful in certain physiological situations [50]. Cellular immune responses are thought to be induced and maintained by regulatory CD4<sup>+</sup> Th1 cells that secrete IL-2 and IFN- $\gamma$ . Local



DCs, which provide a source of costimulatory molecules and cytokines such as IL-12, IL-15, and IL-18, help Th1 cells and CTLs to survive. The findings of an abnormal Th1/Th2 ratio in breast cancer patients with high levels of Th2-type cytokine expression (e.g., IL-4 and IL-10) are supported by clinical research [42]. Most clinical studies have reported abnormal Th1/Th2 ratios in cancer patients. Flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) derived from patients with advanced cancer revealed not only Th1-Th2 imbalances in subsets of PBMCs but also in the capacity for cytokine production. Almost all cancer patients showed changed Th1 or Th2 cytokine profiles, which were normally distinguished by a lower Th1/Th2 ratio [50]. Th cells provide the signals required for preparing MHC class I-limited CD8+ T cells [51]. CTL activation serves as an effecting factor to initiate tumor cell apoptosis. Normally, the resistance of tumor cells is affected by CTL. However, Th1 cells are responsible for CTL initiation. Many reactions can induce Th2 cells movement, as evidence by the various types of malignant growth, with the help of changing serum cytokines [52]. Previous studies have shown that in tumor progression, the balance between T helper type I cells and T helper cell type II occurs in the T3 region. It was shown that an essentially lower level of CD3+ and CD4+ cells resulted in the Th2 cytokine contrast. Different cell populations are changing in such a way that Th1 activity can be profoundly activated. Normally, the activity of IL-10 in malignancy is delivered by type II helper cells, which may change the subsets of T cells. There was some framework for malignant tumor growth to adjust the balance between T helper cells type I and type II, which was partially relieved. Experimentally, it is proved that the increments for Th1 cytokines in Th cells can also diminish the communicating Th2 cytokine increments in the Th cells [49].

## TH CELL-MEDIATED TREATMENT STRATEGIES FOR BREAST CANCER

Several studies have reported a more efficient immune response following the injection of tumor-specific antibodies and vaccines compared to the administration of antibodies or vaccines alone in a transgenic mouse model of breast cancer. These studies developed an endogenous antibody response based on vaccine strategies. Another model demonstrated the successful use of therapeutic agents, such as monoclonal antibodies, against breast tumors. Antibody-mediated immunity is mainly controlled by Th2 cells. Th1 cells play a significant role in the uptake of antigen-presenting cells while Th2 cells are a key factor in high-level humoral immunity. Fallarino et al. [51] reported that types of Th cells reported can precisely the CTL-based immune response. Moreover, the direct infusion of clonal Th cells in tumor-bearing animals activated the CTL anti-tumor response. Th cells can generate various components to directly or indirectly affecting the tumor antigen-specific CTL reaction [46]. In breast cancer, there are 4 major approaches to T cell immunotherapy. Preclinical and clinical research continues to focus on the induction and maintenance of breast cancer-targeted T cell immunity. (A) Active vaccination, in which antigenic tumor associated antigens are transmitted to the patient (in the form of autologous DCs, as peptide in adjuvant, or through a viral vector), stimulating tumor-reactive CD8+ T cell expansion in situ; (B) Adoptive cell transfer, in which tumour antigen-specific T cells are either (i) selected and expanded *in vitro* for re-infusion or (ii) produced from bulk CD8+ populations through genetic modification to induce the expression of tumor-specific T cell receptors or chimeric antigen receptors; (C) Bispecific T cell engagers, which are immunoglobulin domains fusions with specificity for tumours (via a surface-expressed antigen) and T cells (usually via engagement of CD3), resulting to physical tethering of tumors and T cells and subsequent activation of

effector activity by the CD8+ cells, regardless of their nominal antigen specificity; and (D) Checkpoint blockade treatment, which uses humanized antibodies (such as pembrolizumab and nivolumab) to block the expression of death receptors on lymphocytes, shielding them from the deactivation effects or death receptor ligand expression on tumor cells, thus extending the effector activity of infiltrating T cells [47].

## CONCLUSION

STAT1 plays a significant and predictable role in tumor silencing in breast cancer, especially in the early stage of tumor progression; thus, additional studies are needed to determine the mechanisms by which STAT1 influences early stage of breast cancer. Tumor cells often develop modifications to promote tumor resistance; for example, IFN receptor-associated downregulation has been reported in numerous types of tumors. IFNs, a major player in tumor surveillance along with STAT1, are the key factor for transcription mediating IFN-effects. In mammary tumor progression, STAT1 not only influences the failure to respond to IFNs but also controls IFNs. Th cells are key players in adaptive immunity. Technological developments, such as computer algorithms, have helped in understanding how HLA class II peptides activate Th cells. The development of new algorithms has helped to increase data accuracy to 80%–90%. Clinical trials have demonstrated immunity in cancer patients by immunization with HLA-II peptides. Tumor cells themselves has been shown to produce a variety of factors that may alter Th1 or Th2 cytokine levels by either directly affecting T cells or via subsequent interactions with other immune cells, including DCs. Enhancement of tumor growth in the context of Th1/Th2 balance, which regulates cellular and humoral immune responses, is consistent with increasing evidence that cytokines and cellular immunity play a crucial role in tumor rejection or progression. In the end, we can predict that in breast cancer, the recognizable genes that intervene in the suppressive role of STAT1 can be of prognostic value for the use of numerous treatments that boost the activation capacity of STAT1. With this particular set of IFN-inducible genes by STAT1 has been implicated in drug resistance and decreased metastasis-free survival of breast cancer patients, transcriptional profiling of STAT1 may provide useful information about the implementation of efficacious therapies to combat breast cancer.

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