List of 10 Best Publications Prof. Gaurisankar Sa

1. Pati S, Mukherjee S, Guin A, Roy D, Bose S, Dutta S, Saha S, Datta P, Chakraborty J, Sarkar D & **Sa G**. Tumor-associated B-regulatory cell deregulates class-switch recombination to suppress antibody response. ***Cancer Immunology Research*** doi: org/10.1158.2326-6066.CIR-21-1073, 2022. (Impact Factor: 12.02)

   B cells are an essential component of humoral immunity, and their primary function is to mount an antigen-specific antibody response in order to remove invading pathogens. Despite an increase in B cell number, we found that serum-IgG levels were low in breast cancer patients. To solve this conundrum, we used high-dimensional flow-cytometry to analyze the heterogeneity of B cell populations and identified a tumor-specific $CD19^{+}CD24^{hi}CD38^{hi}$ IL10-producing B-regulatory (Breg) cell subset. Albeit IL10 is a defined marker for Breg cells, being an intracellular protein, it is of limited value for Breg cell isolation. Similarly, highly-expressed Breg cell surface proteins CD24/CD38 also impede the isolation of viable Breg cells. These are the major hurdles that prevent many functional aspects of Breg from being revealed. Our transcriptomic analysis identified, CD39-negativity as an exclusive, sorting-friendly surface marker for these tumor-associated Breg cells. The identified $CD19^{+}CD39^{-}IL10^{+}$ B cell branch is suppressive in nature as it limits T-helper cell proliferation, type-1 cytokine production, T-effector cell survival and $CD4^{+}FOXP3^{+}$ Treg cell generation. These tumor-associated immunoregulatory Breg cells were also identified to restrict autologous Tfh cell expansion and IL21 secretion, hence inhibiting germinal transcript formation and AID expression involved in H-chain class-switch recombination. This isotype-switching abnormality eventually hinders B cell differentiation into class-switched memory B cells and subsequent high-affinity antibody-producing plasma B cells, which collectively leads to the dampening of IgG-mediated antibody responses in cancer patients.

2. Sarkar T, Dhar S, Chakraborty D, Pati S, Bose S, Panda AK, Basak U, Chakraborty S, Mukherjee S, Guin A, Jana K, Sarkar DK & **Sa G**. FOXP3/HAT1 axis controls Treg infiltration in tumor-microenvironment by inducing CCR4 expression in breast cancer Key words: Treg cells, tumor-microenvironment, CCR4, FOXP3, HAT1, tumor-infiltration. ***Frontiers in Immunology***, 2022, doi: 10.3389/fimmu.2022.740588 (Impact Factor: 9.4)

   Infiltrating T-regulatory cells in the tumor microenvironment is a key impediment to immunotherapy and is linked to a poor prognosis. We found that tumor-infiltrating Tregs express a higher expression of the chemokine receptor CCR4 than peripheral Tregs in breast cancer patients. CCL22 and CCL17 are released by tumor cells and tumor-associated macrophages, attracting CCR4+ Tregs to the tumor site. The Treg lineage-specific transcription factor FOXP3 changes the CCR4 promoter epigenetically in conjunction with HAT1 to provide a space for FOXP3 binding and activation of the CCR4 gene. To increase CCR4 expression in Tregs, the FOXP3/HAT1 axis is required for permissive (K23 and K27) or repressive (K14 and K18) acetylation of histone-3. In murine breast and melanoma tumor models, genetic ablation of FOXP3 reduced CCR4+ Treg infiltration and tumor size while also restoring anti-tumor immunity. Overexpression of FOXP3, on the other hand, increased CCR4+ Treg infiltration, resulting in a decreased anti-tumor immune response and tumor progression. These findings point to FOXP3 playing a new role in the tumor microenvironment as a transcriptional activator of CCR4 and a regulator of Treg infiltration.

3. Bhattacharyya S, Hossain D Md. S, Mohanty S, Sen GS, Chattopadhyay S, Banerjee S, Chakraborty J, Das K, Sarkar D, Das T & **Sa G.** Curcumin reverses T cell-mediated adaptive immune dysfunctions in tumor-bearing host. Cellular Molecular Immunology 7: 306-315, 2010 (Impact Factor: 24.1)

   Immune dysfunction is well documented during tumor progression and likely contributes to tumor immune evasion. CD81 cytotoxic T lymphocytes (CTLs) are involved in antigen-specific tumor destruction and CD41 T cells are essential for helping this CD81 T cell-dependent tumor eradication. Tumors often target and inhibit T-cell function to escape from immune surveillance. This dysfunction includes loss of effector and memory T cells, bias towards type 2 cytokines and expansion of T regulatory (Treg) cells. Curcumin has previously been shown to have antitumor activity and some research has addressed the immunoprotective potential of this plant-derived polyphenol in tumor-bearing hosts. Here we examined the role of curcumin in the prevention of tumor-induced dysfunction of T cell-based immune responses. We observed severe loss of both effector and memory T-cell populations, downregulation of type 1 and upregulation of type 2 immune responses and decreased proliferation of effector T cells in the presence of tumors. Curcumin, in turn, prevented this loss of T cells, expanded central memory T cell (TCM)/effector memory T cell (TEM) populations, reversed the type 2 immune bias and attenuated the tumor-induced inhibition of T-cell proliferation in tumor-bearing hosts. Further investigation revealed that tumor burden upregulated Treg cell populations and stimulated the production of the immunosuppressive cytokines transforming growth factor (TGF)-b and IL-10 in these cells. Curcumin, however, inhibited the suppressive activity of Treg cells by downregulating the production of TGF-b and IL-10 in these cells. More importantly, curcumin treatment enhanced the ability of effector T cells to kill cancer

cells. Overall, our observations suggest that the unique properties of curcumin may be exploited for successful attenuation of tumor-induced suppression of cell-mediated immune responses.

4. Roy D, Bose S, Pati S, Guin A, Banerjee K, Saha S, Singhal AK, Chakraborty J, Sarkar DK & **Sa G**. GFI1/HDAC1-axis differentially regulates immunosuppressive CD73 in tumor-associated FOXP3+Th17 and inflammation-linked classical Th17 cells. ***Eur J Immunol.*** 2021 Feb 8. doi: 10.1002/eji.202048892 (Impact Factor: 6.69)

   Plasticity between Th17 and Treg cells is regarded as a crucial determinant of tumor-associated immunosuppression. Classically Th17 cells mediate inflammatory responses through production of cytokine IL17. Recently, Th17 cells have also been shown to acquire suppressive phenotypes in tumor microenvironment. However, the mechanism by which they acquire such immunosuppressive properties is still elusive. Here, we report that in tumor microenvironment Th17 cell acquires immunosuppressive properties by expressing Treg lineage-specific transcription factor FOXP3 and ectonucleotidase CD73. We designate this cell as Th17reg cell and perceive that such immunosuppressive property is dependent on CD73. It was observed that in classical Th17 cell, GFI1 recruits HDAC1 to change the euchromatin into tightly-packed heterochromatin at the proximal-promoter region of CD73 to repress its expression. Whereas in Th17reg cells GFI1 cannot get access to CD73-promoter due to heterochromatin state at its binding site and, thus, cannot recruit HDAC1, failing to suppress the expression of CD73.

5. Kajal K, Bose S, Panda AK, Chakraborty D, Chakraborty S, Saha S, & **Sa G**. T-regulatory cell-shed VEGFA induces neo-angiogenesis in tumor micro-environment. ***Cancer Immunol Immunother***, Jan 4. 2021, doi: 10.1007/s00262-020-02808-0. (Impact Factor: 6.10)

   The initiation of new blood vessel formation (neo-angiogenesis) is one of the primary requirements for the establishment of tumor. As the tumor grows beyond a certain size, a hypoxic-condition arises in the inner core of tumor, triggering the release of chemokines, which attract T-regulatory (Treg) cells in the tumor-site. The presence of FOXP3, a lineage-specific transcription factor, expressing Treg cells in various types of tumor implements immunosuppressive and tumor-promoting strategies. One such strategy is the invitation of endothelial cells for neo-vascularization in the tumor site. Here we report that as the disease progresses, Treg cells from breast cancer patients are capable of secreting high-amount of VEGFA. The VEGFA promoter lacks Treg-specific transcription factor FOXP3 binding site. FOXP3 in association with locus-specific transcription factor STAT3 binds to VEGFA promoter to induce its transcription in Treg cells obtained from breast cancer patients. Treg cell-secreted VEGFA induces neo-angiogenesis from endothelial cells under in-vitro conditions. Targeting Tregs in mice with breast tumor reduces tumor growth as well as the level of neo-angiogenesis in the tumor tissue.

6. Chakraborty S, Bhattacharya P, Panda AK, Kajal K & **Sa, G**. Clonal deletion of anti-tumorogenic $IFN\gamma^{hi}FOXP3^{-}CD8^{+}$ Treg cells confine tumor immunosurvillance. ***Immunology Cell Biology***. doi: 10.1111/imcb.12166. 2018 (Impact Factor: 5.85)

   $CD8^{+}$ T-regulatory cells are progressively emerging as crucial components of immune system. The previous report suggests the presence of FOXP3-positive $CD8^{+}$ Treg cells, similar to $CD4^{+}$ Tregs, in cancer patients which produce high levels of IL10 and TGFβ for its immunosuppressive activities. At an early stage of tumor development, we have identified a subset of FOXP3-negative $CD8^{+}CD25^{+}KIR^{+}CD127^{-}$ a Treg-like subset which is essentially IFNγ-positive. However, this early induced $CD8^{+}CD25^{+}CD127^{-}$ T cell subset certainly distinct from the $IFN\gamma^{+}CD8^{+}$ T-effecter cells. This $CD8^{+}CD25^{+}CD127^{-}$ T cells are equipped with other $FOXP3^{-}CD8^{+}$ Treg cell signature markers and can selectively suppress HLA-E-positive T_{FH} cells in autoimmune condition as well as tumor-induced $CD4^{+}$ Treg cells. Contrasting to FOXP3-positive $CD8^{+}$ Tregs, this subset does not inhibit effector T cell proliferation or their functions as they are HLA-E-negative. Adoptive transfer of this early-$CD8^{+}$ Treg-like subset detained tumor growth and inhibited $CD4^{+}$ Treg generation that obstacles the immune surveillance and impairs cancer immunotherapy. At the late stage of tumor development, when $CD4^{+}$ Treg cells dominate tumor-microenvironment, $CD4^{+}$ Tregs mediate the clonal deletion of this tumor-suppressive $FOXP3^{-}IFN\gamma^{+}CD8^{+}CD25^{+}CD127^{-}$ T cells and ensures tumor immune evasion. Our findings suggest that at an early stage of the tumor, this tumor-induced IFNγ-producing FOXP3-negative $CD8^{+}CD25^{+}CD127^{-}$ T cell subset can potentiate immune surveillance by targeting HLA-E-restricted $CD4^{+}$ Treg cells whereas leaving the effector T cell population unaffected, and hence maneuvering their profile can open up a new avenue in cancer immunotherapy.

7. Bhattacharyya S, Mandal D, Sen GS, Pal S, Banerjee S, Lahiry L, Finke JH, Tannenbum CS, Das T & **Sa G**. Tumor-induced oxidative stress perturbs NFκB activity augmenting TNFα-mediated T cell death: Protection by curcumin. ***Cancer Research.* 67**: 362-370, 2007 (Published as *Leading Edge Analysis in* ***Cell 130****:766-768, 2007*) (Impact Factor: 12.71)

   Cancer patients often exhibit loss of proper cell-mediated immunity and reduced effector T cell population in the circulation. Thymus is major site of T cell maturation and tumors induce thymic atrophy to evade cellular immune

response. Here, we report severe thymic hypocellularity along with decreased thymic integrity in tumor-bearer. In an effort to delineate the mechanisms behind such thymic atrophy, we observed that tumor-induced oxidative stress played critical role, as it perturbed NFκB activity. Tumor-induced oxidative stress increased cytosolic IκBα retention and inhibited NFκB nuclear translocation in thymic T cells. These NFκB-perturbed cells became vulnerable to tumor-secreted TNFα-mediated apoptosis through the activation of TRADD-associated FADD and caspase-8. Interestingly, TNFα-depleted tumor supernatants, either by antibody-neutralization or by TNFα-siRNA transfection of tumor cells, were unable to kill T cell effectively. When T cells were over-expressed with NFκB, the cells became resistant to tumor-induced apoptosis. In contrast, when degradation-defective IκBα (IκBα super-repressor) were introduced into T cells, the cells became more vulnerable indicating that inhibition of NFκB is the reason behind such tumor/TNFα-mediated apoptosis. Curcumin could prevent tumor-induced thymic atrophy by restoring the activity of NFκB. Further investigations suggest that neutralization of tumor-induced oxidative stress and restoration of NFκB activity along with the reeducation of the TNFα-signaling pathway can be the mechanism behind curcumin-mediated thymic protection. Thus, our results suggest that unlike many other anti-cancer agents, curcumin is not only devoid of immunosuppressive effects but also acts as immunorestorer in tumor-bearing host.

8. Bhattacharyya S, Mandal D, Saha B, Sen GS, Das T & **Sa G**. Curcumin prevents tumor-induced T cell apoptosis through Stat-5a-mediated Bcl-2 induction**.** ***J Biol Chem***. 282:15954-15964, 2007. [the paper was Press-released by *American Society of Biochemistry & Molecular Biology*] (Impact Factor: 5.59)

   Patients with advanced cancer exhibit multifaceted defects in their immune capacity, which are likely to contribute to an increased susceptibility to infections and disease progression. We demonstrated earlier that curcumin inhibits tumor growth and prevents immune cell death in tumor-bearing host. Here, we report that tumor-induced immuno-depletion involves apoptosis of thymic $CD4^+$/ $CD8^+$ single/double positive as well as loss of circulating $CD4^+$/$CD8^+$ T cells. Administration of curcumin to tumor-bearing animals resulted in restoration of progenitor, effecter and circulating T cells. In fact, tumor burden decreased the expression level of pro-proliferative protein Bcl-2 while increasing pro-apoptotic protein Bax in T cells. Curcumin down-regulated Bax level while augmenting Bcl-2 expression in these cells, thereby, protecting the immunocytes from tumor-induced apoptosis. A search for the up-stream mechanism revealed down-regulation of common cytokine receptor γ chain (γc) expression in T cells by tumor-secreted prostaglandin E-2. As a result, Jak-3 and Stat-5a phosphorylation and to a lesser extent Stat-5b phosphorylation, were also decreased in T cells. These entire phenomena could be reverted back by curcumin, indicating that this phytochemical restored cytokine-dependent Jak-3/Stat-5a signaling pathway in T cells of tumor-bearer. Over-expressed Stat-5a/ constitutively active Stat-5a1*6 but not Stat-5b could efficiently elevate Bcl-2 level and protect T cells from tumor-induced death, whereas C-terminal truncated Stat-$5a_{713}$ over-expression failed to do so, indicating the importance of Stat-5a-signaling in T cell survival. These results, thus, raise the possibility of inclusion of curcumin in successful therapeutic regimen against cancer.

9. Sen GS, Mohanty S, Hossain DM, Bhattacharyya S, Banerjee S, Chakraborty J, Saha S, Ray P, Bhattacharjee P, Mandal D, Bhattacharya A, Chattopadhyay S, Das T, **Sa G.** Curcumin enhances the efficacy of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer. ***J. Biol. Chem.*** 286: 42232-42247, 2011. [*Published as Science News in* ***Nature India, 173,*** November 2011] (Impact Factor: 5.59)

   Breast cancer cells often develop multiple mechanisms of drug resistance during tumor progression which is the major reason for the failure of breast cancer therapy. High constitutive activation of NFκB has been found in different cancers, creating an environment conducive for chemotherapeutic resistance. Here we report that doxorubicin-induced SMAR1-dependent transcriptional repression and SMAR1-independent degradation of IkBα resulted in nuclear translocation of p65NFκB, its association with p300 histone acetylase and subsequent transcription of Bcl-2 to impart protective response in drug resistant-cells. Consistently SMAR1-silenced drug resistant-cells exhibited IkBα-mediated inhibition of p65NFκB and induction of p53-dependent apoptosis. Interestingly, curcumin pre-treatment of drug resistant-cells alleviated SMAR1-mediated p65NFκB activation and hence restored doxorubicin sensitivity. Under such anti-survival condition, induction of p53-p300 cross-talk enhanced the transcriptional activity of p53 and intrinsic death cascade. Importantly, PML-mediated SMAR1 sequestration that relieved the repression of apoptosis-inducing genes was indispensable for such chemo-sensitizing ability of curcumin. Simultaneous decrease in drug-induced systemic toxicity by curcumin might also have enhanced the efficacy of doxorubicin by improving the intrinsic defense machineries of the tumor-bearer. Overall, the findings of this preclinical study clearly demonstrate the effectiveness of curcumin to combat doxorubicin-resistance. We, therefore, suggest curcumin as a potent chemo-sensitizer to improve the therapeutic index of this widely used anti-cancer drug. Taken together, these results suggest that curcumin can be developed into an adjuvant chemotherapeutic drug

10. Das T*, **Sa G***, Hilston C, Kudo D, Rayman P, Biswas K, Molto L, Bukowski R, Rini B, Finke JH & Tannenbaum C. GM1 and TNFα, overexpressed in renal cell carcinoma, synergize to induce T cell apoptosis. ***Cancer Research*** 68**:** 2014-23, 2008. *[*Das T & Sa G both contributed equally]* (Impact Factor: 12.71)

The ability to induce T-cell apoptosis is one mechanism by which tumors evade the immune system, although the molecules involved remain controversial. We found that renal cell carcinoma (RCC)-induced T-cell apoptosis was inhibited by >50% when cocultures were performed with ganglioside-depleted tumor cells, caspase-8-negative lymphocytes, or anti-tumor necrosis factor-alpha (TNFalpha) antibodies, suggesting that tumor gangliosides synergize with signals delivered through TNFalpha death receptors to mediate T-cell killing. The synergy between tumor-derived TNFalpha and the RCC-overexpressed ganglioside GM1 for killing resting T cells is corroborated by studies using purified GM1 and rTNF alpha, which indicate that a 48-hour pretreatment with the ganglioside optimally sensitizes the lymphocytes to a TNFalpha-induced apoptotic death. However, activated T cells, which synthesize TNFalpha themselves, can be killed by exogenous GM1 alone. RelA-overexpressing lymphocytes are protected from GM1 plus TNFalpha-mediated apoptosis, a finding consistent with our previous studies indicating that gangliosides inhibit nuclear factor-kappaB activation. These results are clinically relevant because, similar to T-cells cocultured with GM1-overexpressing RCC lines, T cells isolated from the peripheral blood of patients with metastatic RCC are also heavily coated with that tumor-shed ganglioside. This population of patient cells, unlike T cells isolated from normal donors, is highly susceptible to apoptosis induced by rTNF alpha or by metastatic patient sera, which contain elevated levels of the cytokine. This report thus extends our previous studies by demonstrating that tumor-derived TNFalpha enhances RCC apoptogenicity not only by inducing ganglioside synthesis but also by initiating receptor-dependent apoptosis in T cells in which the nuclear factor-kappaB activation pathway has been inhibited by GM1.