Statement of Research achievements, on which awards have been received by the applicant

Dr. V. V. Kamat Memorial Award for Mid Level Scientist from 'Indian Association for Cancer Research' at RGCB, Thiruvananthapuram, February 2014.

Tumor-Derived Vascular Pericytes Anergize T Helper Cells

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Background: Immune evasion within tumor microenvironment (TME) supports malignant growth, a major obstacle for successful immunotherapy. Multiple cellular components and soluble factors co-ordinate to disrupt protective immune responses in cancer. In such immune disruption, role of the non-hematopoietic stromal cells, particularly pericytes is not studied before. However, in addition to their parenchymal supportive role in cancer establishment and progression, significant immune regulatory role is overlooked.

Methods: Tumor-derived pericytes were isolated by flow sorting from B16 melanoma tumors and normal one from kidneys. Isolated cells were assessed phenotypically by FACS, RT-PCR and WB analysis. The regulatory role of tumor-derived pericytes on CD4 helper T cell functions (activation, proliferation and cytokine production) was then assessed using FACS. The crucial role of tumor pericyte-intrinsic factor, RGS5, was studied using siRNA knockdown or over-expressing plasmid, where as tumor extrinsic factor IL-6 was studied using either recombinant cytokine or neutralizing antibody. The downstream CD4 T cell signaling was studied by WB analysis or by using respective inhibitor of different signaling pathways.

Results: We demonstrate for the first time that tumor-derived vascular pericytes negatively influence $CD4^+$T cell activation and proliferation, thus, promote anergy in recall response to antigen by $CD4^+CD44^+$T cells via RGS5- and IL-6-dependent pathways. Tumor pericytes mainly regulate AKT-mTOR signaling pathway to modulate T cell downstream signaling.

Conclusion: An unexplored role of tumor-derived pericytes in the immune evasion paradigm within the TME is established. Targeting of this cell population is suggestive in the context of designing successful immunotherapeutics for the treatment of cancer.

Best Paper Presentation Award in Symposium of Society of Biological Chemists (India) at CSIR-IICB, April, 2014

Title: **Tumor arrests DN2b to DN3a pro-T cell transition and promotes its conversion to thymic dendritic cells by regulating Notch1 and Ikaros signaling**

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Introduction: Tumor progression leads to severe impairment of intra-thymic T cell differentiation/maturation, leading to the paralysis of cellular anti-tumor immunity. Such suppression is manifested in the erosion of $CD4^+CD8^+$DP thymocytes and an increase in $CD4^-CD8^-$DN early-T cell progenitors. The impact of such changes in the T cell progenitor pool in context of cancer remains elusive. Therefore, present study explored detail mechanism and consequences of tumor induced-blockage in early-T cell development and to know whether NLGP-immunotherapy has any influence.

Methods: Various thymic features during tumor progression were studied in different murine tumor models, with NLGP-therapy. Phenotypic assessment, apoptotic and proliferative status of early-T cell subpopulation were analysed by flow-cytometry. RT-PCR and WB were used to study intra-thymic regulatory factors. Fetal-thymic-organ-culture (FTOC) and IL-$10^{-/-}$ mice were used to decipher the underlying mechanisms.

Results: Tumor progression blocks $Lin^-Thy1.2^+CD25^+CD44^+c\text{-}Kit^{low}$DN2b to $Lin^-Thy1.2^+CD25^+CD44^-c\text{-}Kit^-$DN3a transition, leading instead to DN2-T cell differentiation into dendritic cells (DCs). Thymic IL-10 expression is upregulated, particularly at cortico-medullary junctions (CMJ) under conditions of progressive disease, resulting in the termination of IL-$10R^{high}$DN2-T cell maturation due to dysregulated expression of Notch1 and its target CCR7 (thus restricting these cells to the CMJ). Intra-thymic differentiation of T cells precursors in IL-$10^{-/-}$mice and *in vitro* FTOC revealed that IL-10 promotes the interaction between $Keratin5^+$stromal cells and $Notch1^{low}$ DN2-T cells, thus facilitating these DN2-T cells to differentiate towards $CD45^+CD11c^+MHC\text{-}II^+$ thymic DCs as a consequence of activating the Ikaros/IRF8 signaling axis. Whereas, NLGP-immunotherapy shows normalization of tumor-induced thymic alterations and promotes $CD8^+$ T cell differentiation.

Conclusion: A novel function of thymically-expressed IL-10 in the tumor-bearing host is reported which divert T cell differentiation towards a DC pathway, thus limiting the protective adaptive immune repertoire. Whereas, NLGP-guidance help to generate $CD8^+$ cytotoxic T cell pool in tumor bearer that may ultimately reflect in restriction of tumor growth.

Hiralal Jaju Memorial Best Presentation Award for Mid Level Scientist from 'Indian Association for Cancer Research' at Bose Institute, Kolkata, February 2018.

Targeting CD8$^+$ effector T cells by mesenchymal stem cells and pericytes: An overlooked immune evasion mechanism in cancer

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Background: In context of cancer management, no recent advancement has been more transformative than the rise of immunotherapy. However, discrepancy in laboratory and clinical outcome supported the presence of some hidden factors in masking the immune-therapeutic promises. We have reported earlier that a non-hematopoietic stromal cell (NHSC), pericytes can negatively influence CD4$^+$ helper T cell activity within tumor[a]. Although, CD8$^+$ T cells are main effector cells and its optimum quantity and quality is desired to achieve sustained tumor restriction. But, how pericytes and its progenitor, mesenchymal stem cells (MSCs) influence CD8$^+$ effector T cells remain elusive.

Objective: To elucidate the immune-suppressive mechanisms imparted by two closely-related NHSCs, MSCs and pericytes, towards CD8$^+$ T cells.

Methods: Tumor-MSCs and tumor-pericytes were either isolated by flow-sorting from tumor or generated *in vitro* from C3H10T1/2 cells to ascertain their influence on CD8$^+$ T cell functions (activation, proliferation, cytokine production, cytotoxicity and apoptosis by flow-cytometry). RT-PCR was used to study the fate of T cells. Expression of antigen processing/presentation associated molecules were analysed by RT-PCR and involvement of antigen processing pathways was confirmed using various inhibitors. Flow-cytometry and immunocytochemistry were used to study the phagocytosis and intracellular localization of OVA. The role of RGS5, STAT3 and IL-10 was studied using either Si-RNA based knock-down or in IL-10$^{-/-}$ mice. PROMO 3.0 server-based bio-informatic study was used to identify GAS sequence or the probable binding site of STAT3 that was further validated by ChIP and EMSA.

Results: We are reporting that tumor-MSCs in contact independent way prevent the ability of dendritic cells to promote naïve CD8$^+$ T cell expansion and late-phase effector functions including tumor cell cytotoxicity without affecting activation[b]. Tumor-MSCs secret IL-10 and facilitate binding of STAT3 to a GAS-like motif within cystathionase promoter leading to repression of cystathionase transcription, which generate scarcity of cysteine (required for optimum CD8$^+$ T cell functions). Whereas, tumor-pericytes uptake antigen using phagocytosis and process this antigen preferably via cytosolic pathway to present to CD8$^+$ T cells and prevent activation and effector functions of T cells in contact dependent way. Extrinsic IL-6 and intrinsic RGS5 orchestrates nod-like receptor family member to help tumor-pericytes to acquire 'regulatory antigen presenting cell' like feature and thereby promote antigen specific T cell anergy or deletional tolerance[c].

Conclusion: We have demonstrated for first time that pericytes and MSCs employed completely different mechanisms to suppress CD8$^+$ T cell immunity within tumor microenvironment. Given

the important contribution of NHSCs in immune evasion this study would help in better understanding of tumor biology as well as crafting more successful therapy.