IMMUNOLOGY SECTION

Objectives

To understand tumor-immune interactions in the microenvironment and apply this understanding to:

1. Identify prognostic markers in cancer (cancer progression/chemo-radio-resistance/immunosuppression)

2. Develop immunotherapies that strengthen the immune system and can be used as adjuvants in cancer treatment along with chemo/radio therapies

Low dose radio biology

To understand the

1. Effects of low dose radiation on the immune system

2. Effects of diagnostic/medical exposures on DNA damage response, immune response and antioxidant status in cancer
Immunosuppressive Tumor microenvironment

- Tumor secretes mediators like PGE2
- Immune cells are dysfunctional
- Immune cells secrete T cell suppressive cytokines like TGF-β and IL-10
- Radiotherapy increases secretion of TGF-β

Immunostimulatory Tumor microenvironment

- Immune cells are functional and can directly kill the tumor cells through cell-cell contact
- They can also kill the tumor cells through mediators like nitric oxide.
1. Identify prognostic markers in cancer

Cancer progression

(a) A novel 15 gene signature was identified from macrophage-tumor interactions in breast cancer and has prognostic significance.

- Enrichment genes with significant co-occurrence:
  - TP53, WTAPP1, SLC12A5, PSAT1, ESR1, TPD52, PRKCD

- 8 gene set identified by macrophage-tumor interactions:
  - TNF-α, IL-6, IL-1β, MMP1, MMP9, TGF-β1, TGFβRII, EGFR

- Altered in 63.6% TCGA samples

Cancer radioresistance

(b) TGF-β signaling was increased in radioresistant breast cancer cells resulting in hybrid epithelial-mesenchymal phenotype and enrichment of cancer stem cells.

- Increased TGF-β in radioresistant cells

Immunosuppression

(c) Tumor induced alterations in miRNAs can serve as markers of dendritic cells with lowered immunogenicity.

- miR-155-5p, miR-155-3p and miR-146a-3p upregulated in dysfunctional DC

- miR-362-2-5p, miR-187-3p and miR-142a-3p downregulated in dysfunctional DC

Singh et al., BBA, 2018

Yadav and Shankar, Biomedicine & Pharmacotherapy, 2019
(2.a) Molecules tested as immunotherapeutics in pre-clinical models:

**G1-4A**, polysaccharide from *T. cordifolia* induced killer Dendritic cell phenotype and DC mediated reduction of tumor burden in Lymphoma model

**COX-2 Inhibitor NS-398** abrogated tumor induced DC dysfunction and decreased tumor burden in Lymphoma model

**TGF-β Receptor I inhibitor SB431542** inhibited Breg-Treg axis and reduced tumor burden in Fibrosarcoma model


Pandey et al, Immunology letters, 2017
(2.b) Molecules tested as immunotherapeutics in cell culture systems

PD98059 (MEK inhibitor)

(1) ERK inhibitor abrogated TRAIL induced increase in epithelial-mesenchymal transition in lung cancer cell lines with mutant KRAS

(2) ERK inhibitor abrogated macrophage induced increase in epithelial-mesenchymal transition; cancer stem cells; migration and invasion of breast cancer cells.

(3) Sulforaphane up-regulated NKG2D ligands in lung cancer cell lines thereby activating NK cell-mediated killing.

Pal et al, Cancer microenvironment, 2016

Amin and Shankar, LifeSciences, 2015

(2) FDA approved drug library for identification of COX-2 inhibitors

(1) Epigenetic drug library for identification of inhibitors of T regulatory cell differentiation

c) Molecules being screened as immunotherapeutics
A. Effects of low dose radiation on the immune system (murine model)

Priming Dose
PD (0.1 Gy)

Challenge Dose (2 Gy)
+ConA, 24 h

Radio-adaptive response observed in BALB/c mice and not in C57BL/6 mice

Radio-adaptive response observed in C57BL/6 mice and not in BALB/c mice

PD (0.1 Gy) increased Calcium and RNS in BALB/c and not in C57BL/6 mice

Combination of NO donor and Ca^{2+} ionophore mimic PD and induced RAR in both strains

A. Effects of low dose radiation on the immune system (murine model)

Premkumar and Shankar, IJRB, 2016,
Premkumar et al, IJRB, 2019
B. Biological effect of low and high dose radiation exposure on human peripheral blood mononuclear cells and tissues of cancer patients: a prospective in-vivo study

Principal Investigator: Dr. R. Badwe, Director, TMC

Lead Investigators from BARC: Dr. Birajalaxmi Das, Head, LLRRS; Dr. Bhavani Shankar, Head, Immunology Section

Lead Investigators ACTREC: Dr. Jayant S. Goda, Dr. Supriya J. Sastri, Dr. Sarbani Ghosh Laskar, Dr. Sudeep Gupta, Dr. S. Chiplunkar

Objective: To determine the effects of medical exposures (diagnostic/therapeutic) in blood cells and tissues of normal & cancer patients using multiple endpoints.

PBMC

- DNA damage and repair studies: Analysis of γ-H2AX (positive cells/foci) by flow cytometry and fluorescence microscopy
- Immune response: Cytokine expression by ELISPOT and ELISA
- Gene expression profile: DNA Damage Response and DNA repair genes by RT-qPCR
- Antioxidant status: Lipid peroxidation, lactate dehydrogenase, and levels of Antioxidant enzyme status

Tumor tissues

- Transcriptome sequencing
- Exome sequencing
- miRNA sequencing

Collaborative project with TMH-ACTREC
**FLOW CYTOMETER**

- Provides simultaneous multi-parameter analysis of single cells

**IMAGING CYTOMETER**

- Combines advantages of microscopy and cytometry for high-throughput cellular analysis
- Multichannel digital images of hundreds of thousands of individual cells can be captured within minutes
- Can obtain single-cell morphological and intracellular localization measurements of different cell markers

**ELISPot READER**

- Measures the frequency of cytokine-secreting cells at the single-cell level.
- Each spot corresponds to an individual cytokine-secreting cell.
- Very sensitive and can detect frequencies in \(<10^4\) cells

**Phagocytosis of E.coli Bioparticle by macrophages**

**Proliferation by CFSE dilution in CD4^+ T cells**

**Cell-cell interaction in co-culture**

**Live Dead**

CFSE

PI

**Immune cell mediated tumor killing in co-culture**

**Proliferation of B cells in tumor**

**ELISPot read-out of cytokine secreting cells**

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