Signed details of the excellence in research work for which the Sun Pharma Research Award is claimed, including references and illustrations.

Title: "Deciphering New Mechanisms of the Genesis of Autoimmune Diseases and Cancer"

Project 1. Chronic inflammation is a major factor for genesis for almost all diseases, including autoimmunity, cancer, neurodegeneration, arthritis, heart disease, obesity, and diabetes. Thus, how inflammation is kept under control in cells is of *utmost importance to medical science*. Dr. Chauhan work contributed significantly to this understanding. Importantly, they defined new mechanisms by which **autophagy controls excess inflammation** and protects us from inflammatory diseases. His work has generated new knowledge that could be harnessed to develop new therapeutic formulations to prevent inflammatory diseases and cancer. His research is published in renowned journals of very high repute.

An immunity-related protein named, Immunity-related GTPase M (IRGM) is a medically important gene as its deficiency is shown to be associated with tens of inflammatory and infectious diseases, including Crohn's, tuberculosis, and systemic autoimmune diseases. However, the mechanisms that are employed by IRGM to suppress inflammatory diseases remain elusive for a long time. In a study funded by Wellcome-DBT India alliances, he revealed the mechanism by which IRGM controls the detrimental inflammation to protect us from inflammatory diseases (Mehto et al., 2019, **Molecular Cell**; Jena et al., 2020, **EMBO reports**; Mehto et al., 2019, **Autophagy**; Jena et al., 2020, **Autophagy**; Nath et al 2021, **EMBO reports**).

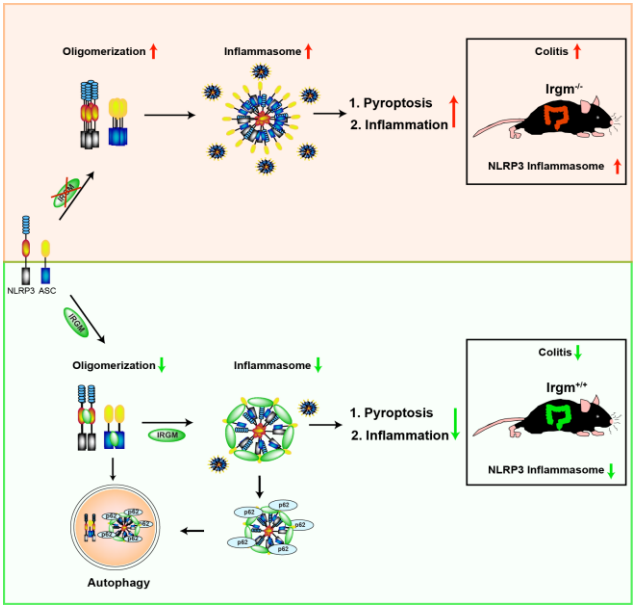

Figure 1. Mechanisms of controlling NLRP3 inflammasomes activation and Crohn's disease by IRGM (Mehto et al., 2019, Molecular Cell)

Mechanistically, they showed that IRGM protein interacts with Inflammasomes components and hinders their oligomerization (Figure 1). Additionally, IRGM mediates autophagic degradation of NLRP3 inflammasomes (Figure 1). Using these two mechanisms, IRGM suppresses NLRP3 mediated inflammation and protects from pyroptosis and inflammatory bowel diseases. This

work was published in ***Molecular Cell*** and ***Autophagy*** Journals. This work got exceptional attention and was highlighted by the Molecular Cell journal in the issue. Since over-activation of inflammasomes is implicated in multiple inflammatory conditions, IRGM could be a potential therapeutic target for these diseases.

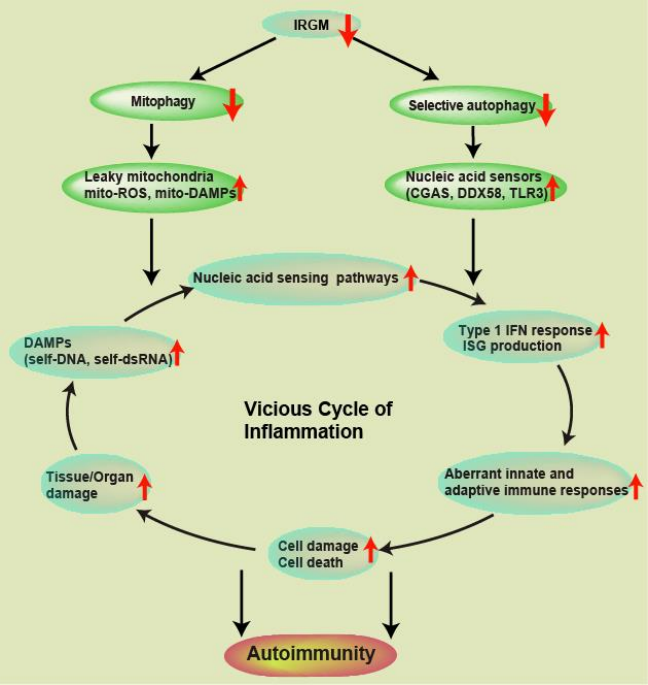

Figure 2. Mechanisms of controlling Interferons and autoimmunity by IRGM (Jena et al. 2020, Autophagy)

The sustained production of type I Interferon (IFN) response can shift beneficial immune systems to self-destructing machinery resulting in tissue damage and autoimmune-like conditions. Therefore, to avoid autoimmune diseases, our body recruits a large number of checkpoints and mechanisms to maintain a delicate homeostatic balance of type I IFNs. Understanding these mechanisms and checkpoints will provide us new targets for therapeutic interventions of autoimmune diseases. Dr. Chauhan lab showed that IRGM protein regulates mitophagy and selective autophagy of nucleic acid sensor proteins (cGAS, TLR3, RIG-I) to keep interferon response under control (Figure 2). This work was published in ***EMBO Reports*** and ***Autophagy*** Journals. IRGM by clearing the defunct mitochondria via mitophagy does not allow the build-up of mtROS and mtDAMPs that are the established stimuli for inducing IFN response (Figure 2). Also, IRGM suppresses the IFN response by degrading nucleic acid sensor proteins via autophagy (Figure 2). In the absence of IRGM, both the stimuli and the sensor are increased in the cells leading to a vicious cycle of inflammation that could result in autoimmune diseases (Figure 2).

Altogether, for the first time, his lab studies demonstrated the reasons why IRGM deficiency in humans is associated with so many autoinflammatory and autoimmune diseases. In addition, they determined new mechanisms by which innate immune homeostasis is maintained in the cells.

Advancing the studies, recently, Dr. Chauhan's group has discovered that depletion of IRGM induces a robust antiviral immune state (MHC-1 antigen presentation, stress granules signaling,

and viral restriction factors) in the cell that could suppress infection of a large number of viruses including SARS-COV2, Zika virus, West Nile virus, Herpes simplex virus, Japanese encephalitis virus, and Chikungunya virus (Nath et al 2021, **EMBO Reports**) (Figure 3)

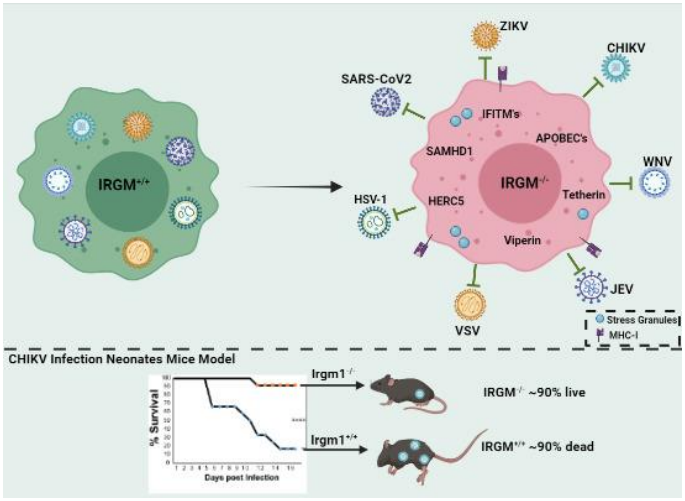

Figure 3. Depletion of IRGM induces a broad antiviral immune response (Nath et al. 2021, EMBO Reports)

Project 2. Dr. Chauhan has also contributed significantly in understanding the mechanisms of cancer progression (Jena et al., 2018 **EMBO J**; Kolapalli et al., 2021, **Cancer Research**; Jena et al., 2019, **Molecular and Cellular Oncology**; Jena et al., 2019, **Autophagy**. He identified a novel cell detoxification mechanism that can suppress oxidative and proteotoxic stresses in cancer cells (Jena et al., **EMBO J**, 2018). In this work, they showed that an E3-Ubiquitin ligase named TRIM16 interacts with NRF2 and controls the conversion of misfolded protein into protein aggregates, and subsequently degrading them by TRIM16-dependent autophagy. Hence, TRIM16 can resolve the oxidative stress-induced cytotoxicity in cancer cells. Thus, targeting TRIM16 in cancer cells could be a new strategy to control cancer progression. Indeed, they showed that upregulation of oxidative stress using arsenic trioxide (a known anti-cancer drug) and depletion of TRIM16 results in regression of tumors in the xenograft mice model. Thus, they described a proof-of-concept for a new combinatorial strategy for therapeutic targeting of cancer (Jena et al., 2019. **Molecular and Cellular Oncology; Cell Stress; Autophagy**).

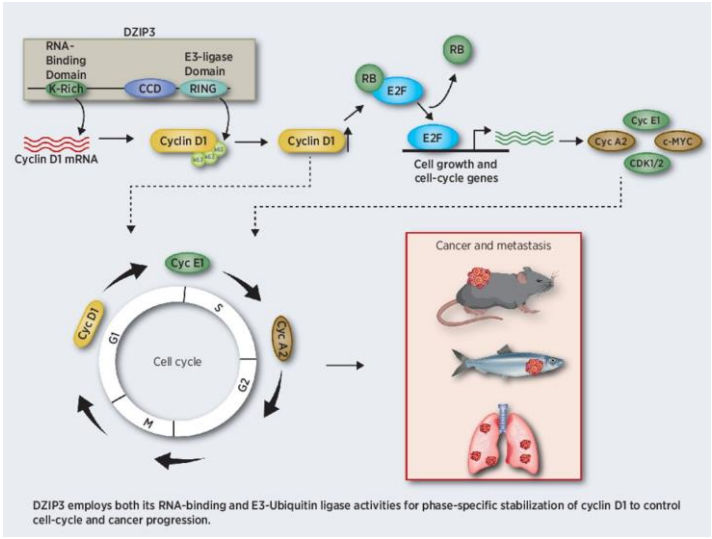

Figure 4. RNA-Binding RING E3-Ligase DZIP3/hRUL138 Stabilizes Cyclin D1 to Drive Cell-Cycle and Cancer Progression

In another very recent work, they showed that a novel RNA binding E3 ligase named DZIP3 is a driver of tumorigenesis and metastasis in mice and zebrafish models by regulating of Cyclin D1-E2F axis (Kolapalli et al., 2021, **Cancer Research**) They showed that DZIP3 utilizes a novel two-pronged mechanism to stabilize Cyclin D1 in cancer cells to drive the cell cycle. First, DZIP3 stabilizes the Cyclin D1 transcripts by binding to its 3′ untranslated region (UTR), and secondly, DZIP3 interacts and increases K63-linked ubiquitination of Cyclin D1 to stabilize it post translationally. DZIP3 was found to be crucial for cancer progression and metastasis in mice and zebrafish. This is for the first time they showed that DZIP3 is amplified in human cancers and could be a new therapeutic target against cancer. This work was highlighted in the issue and was on the cover page of the issue.

Taken together, his work in the last 5 years significantly contributed to the field of inflammation biology and cancer. The work was published in top-tier journals including Molecular Cell, EMBO J, EMBO reports, Cancer Research, and Autophagy, which itself speaks about the quality and importance of the work.

PROJECT-1 WORK IS DETAILED IN THESE PUBLICATIONS:

1. Mehto S,….., Chauhan S*. The Crohn's disease risk factor IRGM limits NLRP3 inflammasome activation by impeding its assembly and by mediating its selective autophagy. **Molecular Cell.** 2019 Feb 7;73(3):429-445.e7. **Corresponding author* (Impact Factor- **17.9**)
2. Mehto S,…. Chauhan S*. IRGM restrains NLRP3 inflammasome activation by mediating its SQSTM1/p62-dependent selective autophagy. **Autophagy**. 2019 Jun 20:1-3. **Corresponding author.* (Impact Factor- **16**)
3. Jena KK, …. Chauhan S*. Autoimmunity gene IRGM suppresses cGAS-STING and RIG-I-MAVS signaling to control interferon response. **EMBO Rep.** 2020 Jul 27:e50051.**Corresponding author* (Impact Factor- **8.8**)
4. Nath P, …., Chauhan S*. IRGM Links Autoimmunity to Autophagy. **Autophagy.** 2020 Aug 19.. PMID: 32813580. **Corresponding author.* (Impact Factor- **16**)
5. Nath P, …Chattopadhyay S*, Chauhan S*. Inhibition of IRGM establishes a robust antiviral immune state to restrict pathogenic viruses. **EMBO Rep**. 2021 Sep 1:e52948. doi: 10.15252/embr.202152948. **Corresponding author*. (Impact Factor- **8.8**)

PROJECT-2 WORK IS DETAILED IN THESE PUBLICATIONS:

1. Jena KK, …..Chauhan S*. TRIM16 controls assembly and degradation of protein aggregates by modulating the p62-NRF2 axis and autophagy. **EMBO J.** 2018 Sep 14;37(18). **Corresponding Author*. (Impact Factor- **11.5**)
2. Jena KK, .. Chauhan S*. TRIM16 governs the biogenesis and disposal of stress-induced protein aggregates to evade cytotoxicity: implication for neurodegeneration and cancer. **Autophagy**. 2019 May;15(5):924-926. **Corresponding author.* (Impact Factor- **16**)
3. Kolapalli SP, ….Chauhan S*, Chauhan S*. RNA binding RING E3-ligase DZIP3/hRUL138 is a novel driver of cell cycle and cancer progression by employing a

unique mechanism to stabilize Cyclin D1. **Cancer Research**, 2021 Jan 15;81(2):315-331. **Corresponding author*. (Impact Factor- **12.5**)

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