## Summary

TRP channels are a group of non-selective cation channels involved in sensory functions, neural regulation, development, growth, immune function, fertility, etc. Till now based on sequence homology, TRP channels are classified into seven subfamilies, out of which six subfamilies, i.e. TRPV, TRPA, TRPC, TRPM, TRPML, TRPP are found in mammals where as TRPN members are restricted to invertebrates and lower organisms. TRP channels are known to be activated by a plethora of exogenous and endogenous chemical ligands as well as physical stimuli such as heat, temperature, pH, osmolarity, etc. The "thermosensing ability" of TRP channels vary from noxious-cold to noxious-hot temperature. Members such as TRPV1, TRPV2, TRPV3 and TRPV4 are "hot-sensitive" ion channel while TRPM8 acts as the "cold-sensitive" ion channel (Zhang *et al*., 2023). Structurally, almost all members of TRP channels share a similar conserved structural feature that includes six transmembrane segments with intracellular C- and N-terminal domains, the "pore-forming" region lying between TM5-TM6. The N- and C-terminus of several TRP channels harbor specific motifs such as ankyrin repeats, CaM-binding domain, TRP-box, etc. which are responsible for binding to several other signaling molecules (Zhao *et al.,* 2021). In this work, the properties of two closely related channels TRPV1 and TRPV3 characterized. These two channels were originated due to gene duplication of TRPV1, but remains genetically linked for 400 million years, sharing similarities yet differing in properties, functions, and regulation. Notably, both TRPV3 and TRPV1 are $Ca^{2+}$-permeable and show thermo-gated behaviour.

TRPV3, plays crucial role in various physiological processes such as thermosensation, transmission of pain signalling, hair growth, skin physiology etc. Point mutations in TRPV3 cause severe genetic disorders in humans such as *Olmsted Syndrome* (termed as OS-mutants). In the present study, it is shown that localization and channel behaviour of TRPV3-WT varies

in response to temperature- and cholesterol-dependent manner. Also localization of TRPV3 into lipid-rafts is varied in OS-mutants when compared to WT condition.

TRPV1 acts as a polymodal channel and is regulated by supramolecular complexes at the plasma membrane composed of membrane proteins, membrane lipids and kinase pathways. In this context TRPV1-R575D represents a special lab-generated mutant that has many features similar to OS-mutants, which shows reduced possibility of interaction with membrane cholesterol. This mutant also shows reduced ligand-sensitivity in control condition along with reduced surface expression, localization in the lipid rafts and induces high cellular lethality, all of these can be fully or partially rescued by adding a TRPV1-specific inhibitor or by introducing another mutation in the next position, i.e. in TRPV1-R575D/D576R. The "ligand-insensitivity" of TRPV1-R575D can also be rescued in certain conditions, such as by chelation of extracellular $Ca^{2+}$ or by reduction of the membrane cholesterol.

Crosstalk between microtubule cytoskeleton with TRPV1 is important for several cellular and physiological processes. Positively-charged residues present at two independent specific tubulin-binding stretch sequences (termed as TBS1 and TBS2) located at the C-terminus of TRPV1 are crucial for tubulin interaction and such sequences have evolutionary origin. The nature of TRPV1-tubulin complex and its functional importance remain poorly understood. In this work, the positively-charged residues within such sequences were mutated to neutral residue. Using live cell $Ca^{2+}$-imaging, different ligands for TRPV1 and also pharmacological modulation of microtubule, the properties of TRPV1-WT, TRPV1-TBS1, TRPV1-TBS2, TRPV1-TBS1+TBS2 were compared.

From the present study, it is hypothesized that regulation of TRPV1 channel function is far more complex to understand. Hence, to understand the interplay of $Ca^{2+}$, cholesterol and Microtubule in regulation of TRPV1, combined mutants harbouring R575D mutation along

with TBS1/TBS2 mutations were generated and ligand sensitivity was studied in different conditions.

Current results also highlight the effect of long-term treatment with Taxol in the context of endogenous expression of TRPV1 and further regulation of subcellular organelles in bone marrow-derived mesenchymal stem cells, relevant in different forms of cancer.

All these studies dissect various factors that can regulate structure-function relationship of TRPV3 and/or TRPV1. This study will also be relevant for developing therapeutic strategies targeting TRP channels in various pathophysiological conditions, especially in the context of chemotherapy-induced neuropathic pain caused by various microtubule-stabilizing chemotherapeutic drugs.