Summary

TRPM8, or Transient Receptor Potential Melastatin 8, is a cold-sensitive cation channel that gets activated with various cooling components and synthetic activators, as well as by low temperature (~25°C). TRP channel was first discovered in *Drosophila* in the year 1969 as a mutant, and 20 years later, the name of this mutant gene was given as trp (Cosens DJ, 1969; Montell C, 1989). To date, around 28 TRP channels have been identified in humans, which are further grouped into six subfamilies, i.e., TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPML (mucolipin), and TRPP (polycystic) (Samanta A, 2018). All TRPs form a tetrameric unit that acts as a cation channel by arranging their transmembrane regions present in the plasma membrane. These channels "sense" various physical and chemical stimuli, e.g., mechanical pressure, thermal changes, and the presence of different endogenous and exogenous chemicals etc. (Lin SY, 2005; Kashio M, 2022).

Among 28 different types of TRP channels, 11 have been identified as "thermosensitive" as these channels sense "cold" or "hot" temperatures and accordingly get activated (Kashio M, 2022). Five TRP channels, i.e., TRPV1, TRPV2, TRPV3, TRPV4, and TRPM2, have been reported as heat-sensing ion channels as these are activated at different temperatures that are either "warm" or "hot." Two TRP channels, i.e., TRPA1 and TRPM8, have been reported to get activated at low temperatures, i.e., at ≤17°C and ≤25°C, respectively, and thus designated as cold-sensitive ion channels. Notably, the "cold-sensitivity" of TRPA1 is still debatable (Chen J, 2013).

Among these ion channels, the work of this thesis is focused on TRPM8, one of the cold-sensitive ion channels. TRPM8 is a thermosensitive cation channel belonging to the transient membrane potential family. TRPM8 is activated by low temperatures (less than 23°C) as well

as by different cooling compounds such as menthol and icilin (Peier AM, 2002). The role of membrane lipids, including PIP_2 and cholesterol, is known to be necessary for the functional regulation of transmembrane proteins (Grouleff J, 2015; Zakany F, 2020; Falkenburger BH, 2010). The role of these channels has extensively been studied in somatic and visceral nociception, where activation of the channel has been followed by the release of various neuropeptides and then leading to neurogenic inflammation (Fakih D, 2020; Ramachandran R, 2013). Apart from its important role in the nervous system, new findings also suggest its role in the immune system and in inflammation (Acharya TK, 2021; Lan X, 2019). Activation and inhibition of these channels have been shown to have various immunological effects in normal conditions as well as in diseased conditions. Hence, in this work, molecular evolutionary aspects of TRPM8, as well as the importance of natural membrane components such as cholesterol, are analyzed.

Both the presence and functions of TRPM8 on the cell membrane are found to be highly dependent on membrane cholesterol, though the exact relationship of TRPM8 with cholesterol is not well understood. Furthermore, TRPM8 has also been found to be the major risk factor in various neurodegenerative diseases, cancer conditions, as well as in various immunological disorders. However, the subcellular organelle-based functions of TRPM8 have not been explored yet. In this thesis work, the role of the endogenous TRPM8 channel has been studied in the F11 and BV2 cell lines, representing neuronal and specialized immune cells, respectively. In addition, this thesis characterizes several subcellular parameters qualitatively and quantitatively as a factor of TRPM8 modulation.

In the first section of this thesis work, the molecular evolution of TRPM8, its different functional domains, amino acids present in the lipid-water-interface regions, and its potential

cholesterol-binding sequence motifs have been analyzed by using multiple sequences from different species belonging to vertebrates. Such analysis suggests that TRPM8 might have co-evolved with membrane cholesterol. In the second section of the thesis work, the F11 cell was used as a model system (DRG neuronal cell line). It was observed that cholesterol reduction leads to the enrichment of TRPM8 at the cell surface. Cholesterol reduction also alters the recycling properties of TRPM8 and intracellular Ca^{2+}-levels due to TRPM8 modulation. In the third section, endogenous expression of TRPM8 in the BV2 (a microglial cell line) cell was observed. The pharmacological modulation of TRPM8 alters the lysosomal pH and lysosomal functions, such as phagocytosis and bacterial clearance within the glial cells. This work establishes a relationship between lysosomal pH and TRPM8 functions in different physiological conditions. In the fourth section, sub-cellular organelle-specific thermosensitive dyes were used. Using two different cellular systems, this study explores further the subcellular thermal spectrum in two different cell types and their alteration as a factor of TRPM8 modulation and/or cholesterol reduction and/or LPS stimulation. In terms of thermal spectrum, different sub-cellular organelles respond differently to cholesterol reduction or LPS stimulation and/or TRPM8 modulation.

Collectively, the data sheds light on the molecular evolution of the TRPM8 ion channel, its relationship with membrane cholesterol, and its cellular functions. At the subcellular organelle level, TRPM8 modulations seem to alter Ca^{2+}-level, pH level, and also sub-cellular organelle-specific temperatures. These findings may be helpful in understanding cellular complexity and membrane components and the role of thermo-sensitive ion channels in such regulations. These findings may have implications for treating different pathophysiological conditions such as neuronal and immunological disorders.