5. Future prospect

In last few years, a lot of information about the TRP channels, especially TRPV1 has been reported, but the exact mechanism of pain sensation via TRPV1 as a whole remains illusive. In an attempt to identify some Ca\(^{2+}\)-sensitive interacting proteins, my work established that TRPV1 interacts with tubulin. Identification of tubulin as a TRPV1 interacting protein and subsequent observations regarding the remodelling of microtubule cytoskeleton by TRPV1 activation opened up multiple new aspects regarding the TRPV1-cytoskeleton cross talk.

First of all, using a number of deletion fragments of TRPV1-Ct, it seems that TRPV1 interacts with tubulin through two short - basic stretches of amino acids. Interestingly, one of these two short basic stretches is highly conserved in all mammals and also in other members of TRPV subfamily. These members reveal interaction with the tubulin and with the microtubules (like MBP-TRPV2-Ct and MBP-TRPV4-Ct were tested positive for tubulin and microtubule binding). Therefore, this short - basic amino acid stretch may represent a novel and unique tubulin-binding motif sequence, which is present in some of the TRPV members. Future study will be conducted to confirm the tubulin-binding site located in all these TRPV members. Specific biotinylated peptides corresponding to these short basic amino acid stretches and mutants will be used to confirm the identity of this tubulin-binding motif.

Secondly, multiple experiments proved that microtubule cytoskeleton is under regulation by TRPV1 and dynamic microtubules disassembled, fragmented due to TRPV1 activation. Preliminary experiments suggest that these changes on microtubules are partly independent on direct Ca\(^{2+}\) influx and may involve enzymatic pathways. There are some indications that activation of CamKII and katanin may be involved in these pathways. Therefore attempt will be taken to clarify the involvement of CamKII and katanin in the TRPV1-activation-mediated microtubule disassembly pathway.

Thirdly, my own experiments and collaborative experiments with Dr Hannes Smith at MDC proved the presence of TRPV1 at the growth cone, in synaptosomes and also in long neurites developed from embryonic DRG explants. These indicate that TRPV1 may be involved in the axonal guidance and path finding process. There are multiple evidences that Ca\(^{2+}\) channels including some of the TRPC channels are involved in similar purpose. Using growth cones and axons developed from DRG-explants, future study will be done to confirm if TRPV1 has any role in the axonal path finding process. This will not only clarify the significance of TRPV1 expression in embryonic stage and for axonal path determination, but
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also shade light on the mechanism by which Ca\(^{2+}\) channels regulate the axonal behaviour via microtubule cytoskeleton.

Furthermore, formation of multiple filopodial structure in non-neuronal cells (like HEK cells) due to over-expression of TRPV1 indicate that TRPV1 may be involved in signalling pathway leading to the formation of filopodial structures. More interestingly, it seems that involvement of TRPV1 in this signalling event does not need the functional full-length channel as over-expression of only N-terminal sequence also results in the similar phenotype. These most likely indicate that N-terminal of TRPV1 harbour the structural information that is important for multiple filopodia formation. While over-expression of multiple and systemic N-terminal deletion constructs in non-neuronal cells will narrow-down the region involved in these process, subsequent pull-down experiment with the short-region will lead to the identification of TRPV1 interacting proteins involved in the process of filopodial structure formation.

Fifth, strong interaction of tubulin with TRPV1 and co-immunoprecipitation of both from native tissue indicate that tubulin is part of a basic constituent of TRPV1-signalplex. Therefore, it was tested if TRPV1 channel properties can be regulated by microtubule cytoskeleton directly. In a collaborative project with Prof. Thomas Boukrowitz at Jena (Germany), a number of drugs, which are known to work on microtubule cytoskeleton, were tested for activity on TRPV1 channel. While, some of the microtubule dynamics-regulating drugs reveal an altered current for TRPV1 to certain extent, taxol\(^{®}\), which is known to causes pain in cancer patients and widely used as a pain model, reveal a sharp alteration of capsaicin currents. Though this observation links the TRPV1 and microtubule cytoskeleton without any doubt in the perspective of functional pain sensation, currently the mode of taxol\(^{®}\) action on TRPV1 remain as a mystery. Future work will be aimed to understand if taxol\(^{®}\) binds directly to the TRPV1 or results in a conformational change on the attach tubulin/microtubule resulting a fast desensitized channel.

Finally, a number of bands corresponding to the proteins which are pulled-down by MBP-TRPV1-Ct from soluble spinal-cord-extract, either in the presence or absence of Ca\(^{2+}\), remain unidentified and/or uncharacterized. Future experiments will lead to identify and characterize all these potential interactors. This will give a lot of information about the TRPV1 regulation and functions, which is important in pain sensation.