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Project Name *
Structure, function and pharmacology of TRP channel TRPV3

Abstract *
Transient receptor potential (TRP) family of ion channels is comprised of 28 members, which based on sequence homology are segregated into 6 subfamilies. Previous studies carried out with the members of TRP channel family have greatly transformed our understanding of sensory modalities of our body, including temperature, pain, and touch. Our research is focused on building up a comprehensive understanding of structure and function of TRP channels family members in physiology and pathophysiology. Currently we are focusing on the TRPV3 member of TRPV (vanilloid) subfamily of TRP channels, which is predominantly expressed in skin keratinocytes and is implicated in cutaneous sensation including thermo-sensation, nociception and itch, in addition to maintenance of the skin barrier, wound healing, and hair growth. Malfunctioning of TRPV3 is associated with a number of skin diseases, including a genodermatosis known as Olmsted syndrome, atopic dermatitis, psoriasis and rosacea. Furthermore, overexpression of TRPV3 is implicated in the development and progression of

colorectal and lung cancer. Therefore, understanding the structure and function of TRPV3 would greatly accelerate the development of novel drugs to treat TRPV3-associated diseases. TRPV3 is activated at innocuous temperatures (~33°C and higher) as well as by natural compounds such as camphor, carvacrol, eugenol and thymol, as well as by the small synthetic compound 2-aminoethoxydiphenyl borate (2-APB), which has recently been shown to suppress tumor growth and invasiveness. In our efforts towards TRPV3 structure determination, we screened a number of TRPV3 orthologues and discovered mouse TRPV3 as a promising target for our structural studies. Recently, we used cryo-EM to determine the first structures of mouse TRPV3 in the closed and agonist 2-APB bound open states (Singh et al, NSMB 2018). Comparing the structures of TRPV3 in the closed and open states, we deduced the dramatic conformational changes associated with TRPV3 gating. More specifically, our structures explain how 2-APB, a small molecule with anti-cancer properties, binds to and interacts with TRPV3. Next we want to better understand pharmacology of this channel in order to design small molecules with enhanced efficacy and affinity to inhibit TRPV3. Currently, our structural efforts are focused on understanding of how the small, naturally occurring molecules, including camphor, carvacrol, eugenol and thymol, bind to and modulate gating of TRPV3 (Figures 1-3). Our studies will not only explain the molecular mechanism of action of natural compounds on TRP channels but provide a spring board to design novel antagonists of TRPV3 with higher efficacy and affinity that might eventually become therapeutically useful drugs.

Scientific Impact *

Structures of TRPV3 in complex with camphor, carvacrol, eugenol and thymol will uncover structural bases of TRP channel regulation by small naturally occurring compounds and will provide information important for the design of new compounds that can eventually become drugs for treatment of devastating human diseases.

Scientific Feasibility *

We have recently solved structures of TRPV3 in complex with 2-APB, a small molecule that is close in size to camphor, carvacrol, eugenol and thymol. Preliminary cryo-EM data collected for TRPV3 in the presence of camphor shows promise and suggest feasibility of our research goals.

Technical Feasibility *

Previously, the structure of TRPV3 in complex with 2-APB was solved by cryo-EM based on a single 2-day collection using Krios microscope. For new structures, we will use similar protein/cryo-EM sample preparation. We expect that each new TRPV3 complex with small natural compound will require one 2-day Krios collection.

Resources Requested *

We request one 2-day Krios collection (per one TRPV3 complex with a small natural compound, first camphor).

Geographic/Demographics *

Currently, Columbia University has access to 50% of Krios 3 time at NYSBC, a share of time at other NYSBC instruments, and F30 Polara and F20 at the Columbia Medical campus (the last two are old instruments that are often non-functional). Columbia University is currently installing new instruments but we don't have an access to them yet.

Supplementary Information *

The proposal may have up to 1 page of data and figures for supporting material.
All attachments should be in pdf format.

All key personnel named in the application should upload a NIH biographical sketch.