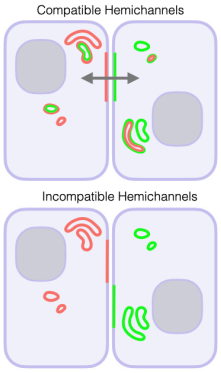

A platform for high-throughput quantification of gap junction hemichannel docking

Unmet Need

Gap junctions are transmembrane channels that facilitate for cell-cell communication. They play a crucial role in normal cellular physiology and their dysfunction or misregulation is associated with many human diseases. Gap junctions are made up of proteins within the 21- member connexin (Cx) family. Various Cx isoforms and mutations are thus associated with disease and research tools are required to understand how Cx isoforms regulate gap junction compatibility, docking and permeability. Existing fast and accessible methods depend on permeability of gap junctions to small molecule dyes however few dyes are gap junction permeant and these dyes are typically connexin isoform specific. The current gold standard method, dual-patch clamp electrophysiological measurement, is low-throughput, expensive and requires high technical skill. The global market for reagents and tools for cell-based assays was valued at $21.3 billion in 2018 and is growing at a CAGR of 12.9%, expected to reach $39 billion in 2023. Thus, there is a need for a high-throughput, broadly applicable screening method to address the impact of disease-relevant Cx protein mutations and isoforms on gap junction formation and function.

Technology

Duke inventors have developed a flow cytometry-based technique for the evaluation of cellular gap junctions. This is intended to be used as a research tool for the study of how different connexin isoforms and mutations affect function of gap junctions and contribute to human disease progression. Specifically, this method entitled FETCH (flow cytometry enabled tracking of connexosomes in HEK cells), is a two-component system consisting of the fluorescent reporters and HEK cell lines for flow cytometry and a software code used for analysis of data. In the execution of the method, the HEK 293FT cell lines are transiently transfected with fluorescently labeled, C-terminally fused connexin constructs. Following co-culture of cell lines with two different fluorescent markers, flow cytometry generates fluorescence profiles of the individual cells allowing for quantitative analysis of fluorescence exchange between cells mediated by the formation and turnover (internalization) of gap junctions. The accompanying software code generates a FETCH score that can be used as a standard metric to evaluate gap junction function. This has been demonstrated in the HEK 293FT cellular background *in vitro* with multiple different connexin isoforms and disease relevant connexin mutant proteins.

Other Applications

In addition to the characterization and understanding of major aspects of Cx isoforms and mutants in physiology and disease, this method can bed used to engineer desirable docking properties into Cxs and generate completely novel gap junctions.

Advantages

- High-throughput system allowing for potential analysis of 96 samples at a time using a flow cytometry plate reader system
- Broadly applicable to all connexin isoforms and mutations
- Easy to perform, does not require high technical skill
- Can be performed using any flow cytometer

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Psychiatry & Behavioral Sciences (Dept. & CRU)

Publication(s)

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External Link(s)

• From the lab of Dr. Kafui Dzirasa