**Ca\textsuperscript{2+} Entry & Maintenance of Oscillatory Ca\textsuperscript{2+} Signals in Human Carcinoid Cell Lines**

Tetyana Zhelay, Sasi Arunachalam, and David Giovannucci

Dept. of Neurosciences, University of Toledo College of Medicine, Toledo, OH USA

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**BACKGROUND**

Calcium entry following exogenous activation (ER) Ca\textsuperscript{2+} depletion and store-operated Ca\textsuperscript{2+} channel activation requires proliferation, migration, and anchorage in some cancer cells including those of the foregut. These cells express a variety of store-operated Ca\textsuperscript{2+} channel (SOCE) including key components of more sophisticated Ca\textsuperscript{2+} entry (SOCE). The ER Ca\textsuperscript{2+} sensor STIM1 and the plasma membrane channel ORAI1 (Orai1) and Orai3 (Orai3). However, the role of SOCE is unclear in the carcinoid cell lines and its role for the establishment of tumor-like structures in an organ-slice model is activated by the induced interaction between ER Ca\textsuperscript{2+} sensor STIM1 and ORAI1 e106A and Orai3 e81A was performed using the organ-slice model.

**METHODS**

1. Carcinoid cell lines express mAChRs

   A. Representative traces of Ca\textsuperscript{2+} dynamics induced by CCh in the presence of various concentrations extracellular Ca\textsuperscript{2+} concentrations or SOCE inhibition (BTP2)

2. Muscarinic activation of Ca\textsuperscript{2+} entry (SOCE)

   A. Functional assessment of SOCE by over-expression of wild type or dominant negative ORAI1 protein enhanced Ca\textsuperscript{2+} oscillations in human carcinoid cancer cell lines and support a role for ORAI1 in formation of tumors in mouse liver

3. Relationship of GPCR activation and SOCE

   A. Ca\textsuperscript{2+} store content

4. Role of ORAI in CCh-evoked entry and maintenance of Ca\textsuperscript{2+} oscillations

   A. Representative traces for BON and H727 carcinoid cell lines. A. Examples of CCh-evoked Ca\textsuperscript{2+} oscillations and atropine magnitude of influx (measured by barium entry) to CCh-induced signal mass.

5. Tumor formation in mouse liver requires ORAI 1

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**CONCLUSIONS**

- mAChR activation in BON and H727 cells evoked Ca\textsuperscript{2+} oscillations in a dose- and extracellular Ca\textsuperscript{2+} dependent fashion.
- Inhibition of Ca\textsuperscript{2+} entry, silencing of ORAI or STIM or over-expression of a dominant negative ORAI significantly diminished Ca\textsuperscript{2+} oscillations, whereas over-expression of wild-type ORAI protein enhanced Ca\textsuperscript{2+} oscillations.
- In addition, BON cells deficient in ORAI were unable to reliably form tumors in our organ slice model.
- These data indicated that ORAI is required for agonist induced Ca\textsuperscript{2+} entry, maintenance and frequency of Ca\textsuperscript{2+} oscillations in human carcinoid cancer cell lines and support a role for ORAI1 in formation of tumors in mouse liver

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