# B12

## Calcium entry and Maintenance of Oscillatory $Ca^{2+}$ Signals in Human Carcinoid Cell Lines.

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**Background:** Cytosolic $Ca^{2+}$ oscillations evoked by G protein-coupled receptor activation can regulate cell cycle, migration and apoptosis in some cancer cells including those of neuroendocrine phenotype. Typically, oscillatory signals depend on release of $Ca^{2+}$ from internal stores as well as entry through plasma membrane channels. However, little is known regarding the role and molecular underpinnings of this $Ca^{2+}$ entry in carcinoid cell lines. In the current study we elucidated the role of STIM and ORAI (key components of a $Ca^{2+}$ permeable channel that we have previously identified in carcinoid cell lines) in agonist induced $Ca^{2+}$ entry.

**Methods:**
BON and H727 cell lines were loaded with $Ca^{2+}$ sensitive dyes and monitored by fluorescence imaging. Carbachol (CCh) application was used to activate $Ca^{2+}$ oscillations and pharmacological inhibition, targeted gene silencing and over expression techniques were used to probe the effect of ORAI-mediated $Ca^{2+}$ entry on oscillatory $Ca^{2+}$ signals. In other experiments, multi-photon microscopy was used to assess the role of ORAI on the kinetics of tumor formation in cultured mouse liver slices.

**Results:** Activation of muscarinic acetylcholine receptors in BON and H727 cells evoked $Ca^{2+}$ oscillations in a dose- and extracellular $Ca^{2+}$-dependent fashion. Inhibition of $Ca^{2+}$ entry, silencing of ORAI or STIM or over-expression of a dominant negative ORAI significantly diminished the frequency and maintenance of $Ca^{2+}$ oscillations, whereas over-expression of wild-type ORAI protein enhanced both frequency and amplitude of $Ca^{2+}$ oscillations. In addition, BON cells deficient in ORAI were unable to reliably form tumors in our organ slice model.

**Conclusions:** These data indicated that ORAI is required for agonist induced $Ca^{2+}$ entry, maintenance and frequency of $Ca^{2+}$ oscillations in human carcinoid cancer cell lines and support a role for ORAI 1 in formation of tumors in mouse liver.