436 Assessment of calcium responses induced by the transient receptor potential cation channel subfamily V member 4 (TRPV4) activator GSK1016790A in MDA-MB-468 breast cancer cells using automated epifluorescence microscopy.

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Introduction. The transient receptor potential cation channel subfamily V member 4 (TRPV4) is elevated in the basal molecular subtype of breast cancer (Peters et al, 2017). These breast cancers have poor prognosis and significantly overlap with the triple negative breast cancers. TRPV4 appears to contribute to the migration potential of breast cancer cells (Lee et al, 2017). However, the consequences of pharmacological activation of TRPV4 using the TRPV4 activator GSK1016790A have not been fully explored, particularly in the context of single cell Ca^{2+} imaging.

Aims. To assess temporal and spatial changes in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) induced by the TRPV4 activator GSK1016790A in MDA-MB-468 basal breast cancer cells.

Methods. MDA-MB-468 cells were plated onto 96-well microplates and loaded with the Ca^{2+} sensitive indicator Fluo-4 or Fura-2. Fluorescence changes induced by 0, 1 or 100 nM of GSK1016790A were detected using an automated epifluorescence microscope (ImageXpress). Image segmentation analysis was used to assess changes in $[Ca^{2+}]_{CYT}$ as assessed by Fluo-4, and ratiometric imaging was used to assess relative levels of $[Ca^{2+}]_{CYT}$ in Fura-2 loaded MDA-MB-468 breast cancer cells.

Results. MDA-MB-468 breast cancer cells exhibited spontaneous $[Ca^{2+}]_{CYT}$ oscillations. GSK1016790A at 100 nM induced pronounced, rapid and sustained increases in $[Ca^{2+}]_{CYT}$ in MDA-MB-468 breast cancer cells. Pronounced single cell heterogeneity was observed in $[Ca^{2+}]_{CYT}$ changes.

Discussion. These studies provide further evidence that MDA-MB-468 cells express functional TRPV4 channels and suggest that there may be significant heterogeneity in MDA-MB-468 breast cancer cell responses to TRPV4 activation.

Peters AA et al (2017) Oncogene (in press)
Lee WH et al (2017) Oncogenesis. 6:e338.¶

437 PAR_1 and PAR_2 open TRPV4 with conserved signalling pathways

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Introduction. We have previously shown that the pro-inflammatory G-protein coupled receptor, protease-activated receptor 2 (PAR_2) signals to and opens TRPV4 channels in HEK293 cells (Poole et al., 2013). We identified molecules which transduce signals from PAR_2 to TRPV4 using siRNA inhibition and identified signalling molecules which include heterotrimeric G-proteins, phospholipases and protein kinases (Darby et al., unpublished). In this study, we investigated whether any of the identified siRNA targets also transduce signals from PAR_1 to mediate TRPV4 opening.

Aims. To determine if PAR_1 and PAR_2-dependent opening of TRPV4 in HEK293 cells shares signalling mechanisms.

Methods. Parental HEK293 cells and HEK293 cells stably expressing human TRPV4 were transfected with Dharmacon SMARTpool siRNAs and each well was subsequently assayed for PAR_1-dependent opening of TRPV4 using a fura-2am fluorescence ratiometric intracellular calcium ($[Ca^{2+}]_i$) assay. Cells were injected with PAR_1 activating peptide (TFFLR-NH_2, 50 µM) followed by the selective TRPV4 agonist (GSK101067A, 30 nM), 85 s later. The area under curves from 50 – 90 s was compared using one-way ANOVA with Sidak's post hoc t-test.

Results. In parental HEK293 cells, PAR_1 activation transiently increased $[Ca^{2+}]_i$ (area = 12 ± 3). Functional expression of human TRPV4 caused a sustained increase of $[Ca^{2+}]_i$ (area = 43 ± 6) which was abolished by the TRPV4 antagonist (HC067047, 1 µM) (area = 9 ± 4). siRNA knockdown of $G\alpha_{13}$ and $G\gamma_8$ significantly ($p < 0.05$) inhibited PAR_1-dependent opening of TRPV4 reducing area by 52 ± 8% and 39 ± 7% respectively. Inositol-tetrakisphosphate 1-kinase (ITPK1), mitogen-activated protein kinase 13 (MAPK13) and lysine deficient protein kinase 4 (WNK4) also reduced area by 44 ± 12%, 69 ± 9% and 39 ± 9% respectively. Phospholipase A_2 group 4 (PLA_2G4) reduced the area by 54 ± 12%.

Discussion. Activation of GPCRs results in simultaneous activation of parallel signalling pathways. Therefore, inhibition of TRPV4 opening by a specific siRNA pool is an indication that the target protein contributes to PAR_1-dependent opening of TRPV4. Like PAR_2 receptors, PAR_1 receptors were found to couple to TRPV4 through heterotrimeric G-protein subunits, $G\alpha_{13}$ and $G\gamma_8$, PLA_2G4, and kinases ITPK1, MAPK13 and WNK4 in HEK293 cells.

Poole et al. (2013) J Biol Chem, 288:5790-5802¶