Microtopographical features recruit RhoA/ROCK through TRPV1 channels to direct cell and neurite growth

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Abstract

• Development of neural circuits requires targeting of axons and dendrites.
• Micropatterned polymers were used to identify mechanisms by which growth cones translate biophysical cues into directed neurite regeneration.
• We previously described the role of RhoA in directing growth, and here we demonstrate the role of Rho associated kinase (ROCK) and TRPV1 channels as mediators of cell and neurite responses to surface topographical cues.
• These studies demonstrate that biophysical features recruit similar signaling pathways as chemorepulsive guidance cues to direct neurite growth.

Methods

Figure 1. Photopolymerized micropatterns direct neurite growth. A. Mixture of HMA, HDDMA and photoinitiator is UV cured beneath photomasks for patterned samples (upper row), or with glass microscope slides for unpatterned samples (lower row). B-D. Representative SEM micrographs of top-down (B), cross-section (C), and angled cross-section (D) views of micropatterned substrates. E-F. SGN neurites grow in random directions on unpatterned substrates (E) but orient to the direction of the pattern (horizontal) on patterned surfaces (F).

Results

Figure 2. ROCK1 and 2 activity facilitate neurite alignment to micropatterned surfaces. A-C. pROCK immunofluorescence intensity is higher in SGN neurites on micropatterned substrates (C) compared to those on unpatterned surfaces (B). D-I SGN alignment is significantly reduced in cultures treated with ROCK inhibitors H1152 or Y27632. J. Transfection of spiral ganglion cultures with DsiRNA oligonucleotides targeting ROCK1 or ROCK2 reduces mRNA expression as determined by real-time RT-PCR compared to cultures transfected with a scrambled, non-targeted oligonucleotide. K-N. Neurite alignment in cultures transfected with ROCK1 DsiRNA or ROCK2 DsiRNA is decreased compared to alignment when treated with a scrambled oligonucleotide on micropatterned substrates.

Figure 3. TRPV1 mediates neurite and 3T3 cell alignment to micropatterned surfaces. A. SKF96365, Ruthein Red and gentamicin decrease SGN alignment on micropatterned substrates. B-D. Expression of TRPV1 in SGN growth cones immunostained with anti-NF200 (C, green) and anti-TRPV1 (D, red) antibodies with combined labeling (E). F. Transfection of cultures with TRPV1-targeted DsiRNA oligonucleotides decreases SGN alignment compared to transfection of a scrambled oligonucleotide. G,H. Images of SGN alignment in cultures treated with scrambled (G) or TRPV1-targeted DsiRNA oligonucleotides (H). I. Transfection of 3T3 cells with a TRPV1 expression vector increases 3T3 alignment compared to cells transfected with an empty plasmid. J,K. Images of 3T3 cells cultured on micropatterns and co-transfected with empty and GFP expression plasmids (J) or TRPV1 and GFP expression plasmids (K).

Conclusion

• Modification of micro-features in artificial platforms is able to achieve cellular responses and integration with native tissue.
• Gradual transitions created by photopolymerization were utilized to identify intracellular signals responsible for neurite alignment to micropatterns.
• Transition slopes of topographical features activate intracellular signals including RhoA and ROCK to guide neurite growth
• TRPV1 channels are necessary for topographical guidance by contributing to RhoA activation.

References

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