

## RACK 1 associated with focal adhesions alters the cells sensitivity to contact guidance.

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**INTRODUCTION:** Through the use of firstly microtopography and then nanotopography, it has been shown that all cell types tested experience contact guidance. Typically, the most extreme cases have been demonstrated through the use of grooves, along which the cells align.

Filopodial sensing (a nanoscale form of contact guidance) and whole cell guidance are mechanisms of cellular motility and are thus likely to involve focal adhesions and the cytoskeleton. In this report, we focus on RACK1, a part of the focal adhesion. Using nanodepth grooves to align cells of three types: NEO (normal), RACK1 overexpressing (RACK<sup>+</sup>) and neo cells treated with an antisense oligo for RACK1 (hence express little RACK 1, RACK<sup>-</sup>), we have observed the effects of RACK 1 on contact guidance.

**METHODS:** Grooved substrates were fabricated in silicon by photolithography. The grooves were 10  $\mu\text{m}$  wide and 200 nm deep. The master substrates were used as a stamp to hot-emboss the grooves in to polycaprolactone (PCL) sheets.

As described in the introduction, NEO, RACK<sup>+</sup> (plasmid transfection) and RACK<sup>-</sup> (scrape loading) MCF-7 cells were cultured on the grooves and planar PCL for 3 days before fixation. After fixation, cells were stained for vinculin, actin and tubulin and observed by fluorescence microscopy.

As well as morphological observation of adhesions and cytoskeletons, percentage of aligned cells was

calculated in order to measure degree of contact guidance.

**RESULTS & DISCUSSION:** Compared to NEO, RACK<sup>+</sup> cells had numerous focal adhesions, well defined actin cytoskeleton and were poorly aligned. Compared to NEO, RACK<sup>-</sup> cells had few focal contacts, only peripheral actin, but were well aligned.

It appears that increasing adhesion formation through up-regulation of RACK 1 leads to decreased motility and hence contact guidance.

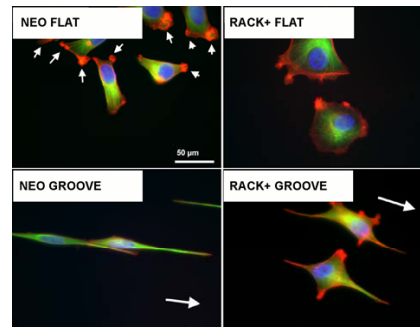


Fig. 1: RACK<sup>+</sup> cells on flat and grooved materials – comparison to NEO.

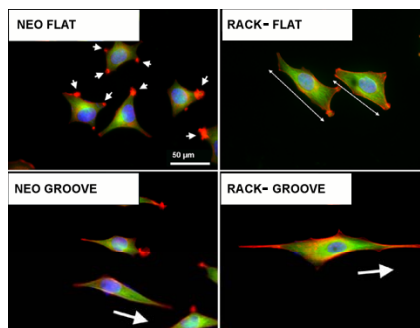


Fig. 2: RACK<sup>-</sup> cells on flat and grooved materials – comparison to NEO.

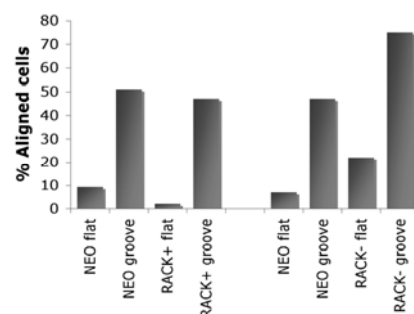


Fig. 3: % Aligned cells for each treatment.

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