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Article

RAC1B Suppresses TGF-β1-Dependent Cell Migration in Pancreatic Carcinoma Cells Through Inhibition of the TGF-β Type I Receptor ALK5

Supplementary Material

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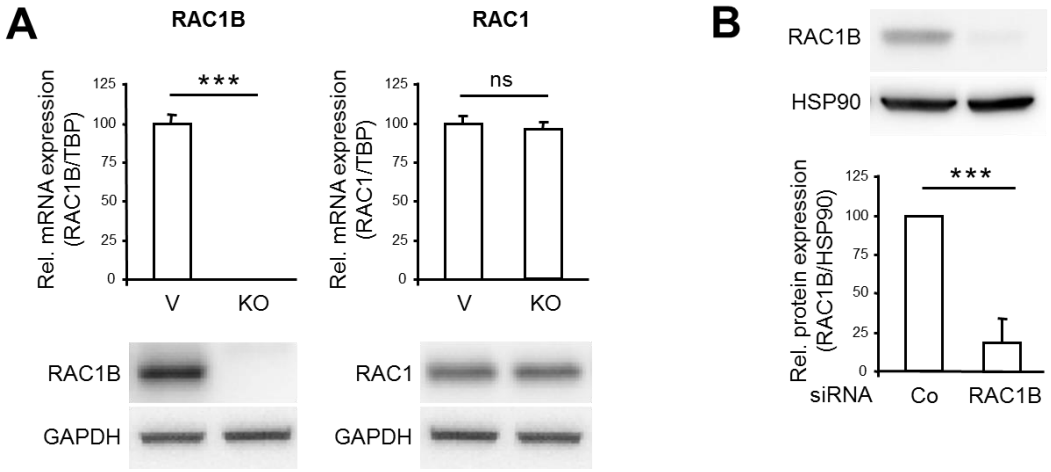

Figure 1. Quantification of RAC1B expression in Panc1-RAC1B-KO and Panc1-RAC1B-KD cells. (**A**) Panc1 cells in which exon 3b of *RAC1* had been deleted by CRISPR/Cas technology (Panc1-RAC1B-KO) were subjected to qPCR and immunoblot analysis for RAC1B, and RAC1 as control. The qPCR data (graphs) were normalized to those for TBP and are the means ±SD from three parallel wells. Below the graphs the corresponding immunoblots are shown. GAPDH was used as a loading control. (**B**) Panc1 cells were transiently transfected twice (on two consecutive days) with 50 nM of either control siRNA (Co) or RAC1B siRNA (RAC1B). Forty-eight h after the second round of transfection cells were lysed and subjected to immunoblot analysis for RAC1B, and HSP90 as a loading control. The graph underneath the blot shows quantification from densitometric analyses. Signal intensities for RAC1B were normalized to those for HSP90 and represent the mean ±SD from six independent experiments. The asterisks indicate significance; ns, not significant. For a detailed description of the generation of Panc1-RAC1B-KO and –KD cells see Methods section.

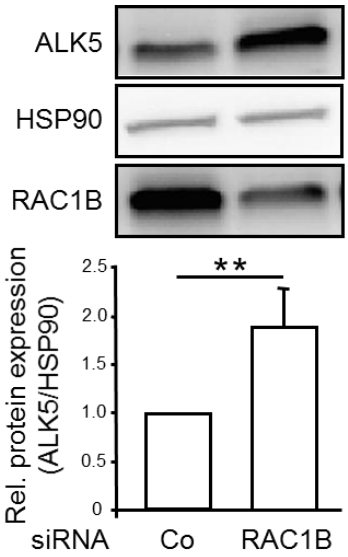

Figure 2. Effect of RAC1B KD on ALK5 expression in the PDAC-derived cell line Colo357. Colo357 cells were transfected twice (on two consecutive days) with 50 nM of either irrelevant control siRNA (Co) or siRNA specific for RAC1B (RAC1B). Forty-eight h later cells were subjected to immunoblotting for ALK5, HSP90 as a loading control, and RAC1B as a control for transfection efficiency. The graph underneath the blot shows quantification from densitometric analyses. Signal intensities for ALK5 were normalized to those for HSP90 and represent the mean ±SD from three independent experiments. The asterisks indicate significance (student's *t* test).

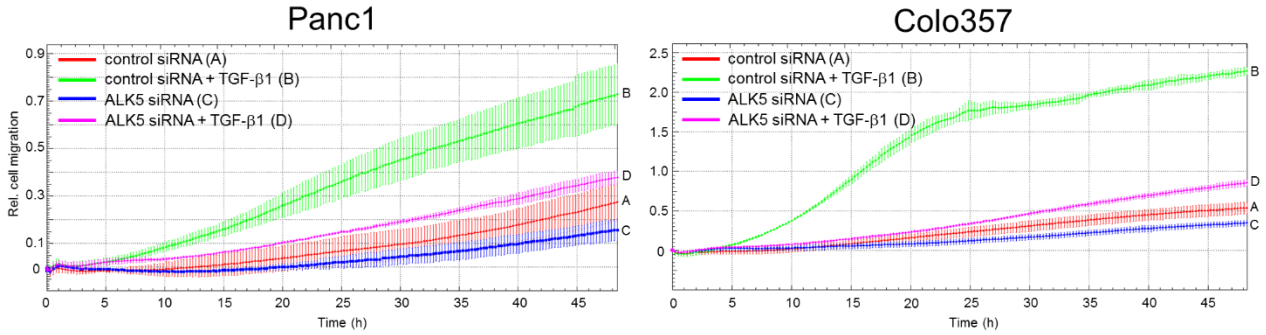

Figure 3. Effect of ALK5 KD on TGF-β1-induced migration of Panc1 and Colo357 cells. Panc1 or Colo357 cells were transfected twice with 50 nM of either control siRNA or ALK5 siRNA. Forty-eight h after the second transfection, cells were processed for migration assay on the xCELLigence platform. Immediately before the start of the assay one half of the cells received 5 ng/mL TGF-β1. Data are from one representative experiment and are the mean ±SD from 3–4 wells per condition. Differences between Panc1 cells + ALK5 siRNA + TGF-β1 (magenta curve, tracing D) and Panc1 cells + control siRNA + TGF-β1 (green curve, tracing B) are significant at 07:45 and all later time points. Differences between Colo357 cells + ALK5 siRNA + TGF-β1 (magenta curve, tracing D) and Colo357 cells + control siRNA + TGF-β1 (green curve, tracing B) are significant at 06:30 and all later time points. Successful inhibition of ALK5 protein expression was verified by immunoblotting (not shown). For functional validation of the ALK5 siRNA see Figure 1A.

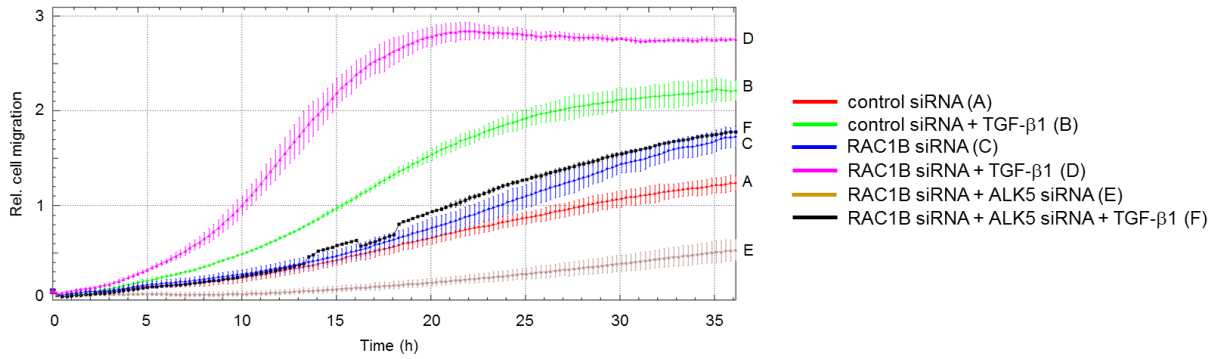

Figure 4. Effect of RAC1B KD, ALK5 KD, and combined RAC1B/ALK5 KD on TGF-β1-induced migration of Colo357 cells. Colo357 cells were transfected twice with either 50 nM of control siRNA, 25 nM RAC1B siRNA+ 25 nM control siRNA, or 25 nM RAC1B siRNA + 25 nM ALK5 siRNA. Forty-eight h after the second transfection, cells were processed for migration assay on the xCELLigence platform. Immediately prior to the start of the assay one half of the cells received 5 ng/ml TGF-β1. Data are from one representative experiment and are the mean ± SD from 4 wells per condition. Differences between Colo357 cells + RAC1B siRNA + ALK5 siRNA + TGF-β1 (black curve, tracing F) and Colo357 cells + RAC1B siRNA + TGF-β1 (magenta curve, tracing D) are significant at 04:30 and all later time points. Successful inhibition of RAC1B and ALK5 protein expression was verified by immunoblotting (data not shown).

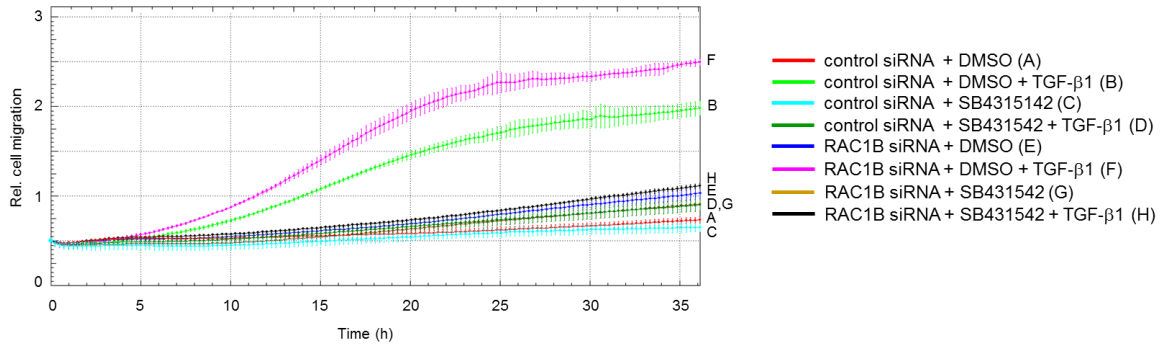

Figure 5. Effect of pharmacologic inhibition of the ALK5 kinase activity on TGF-β1 and RAC1B KD-induced migration of Colo357 cells. Colo357 cells were transfected twice, on two consecutive days, with 50 nM of either control siRNA or RAC1B siRNA. Forty-eight h after the second transfection, cells were processed for migration assay on the xCELLigence platform. Immediately prior to the start of the assay one half of the cells received 5 ng/ml TGF-β1 along with either the ALK5 kinase inhibitor SB431542 (5 μM) or solvent (dimethyl sulfoxide, DMSO). Data are from one representative experiment (three performed in total) and are the mean ± SD from 4 wells per condition. Differences between Colo357 cells + RAC1B siRNA + SB431542 + TGF-β1 (black curve, tracing H) and Panc1 + RAC1B siRNA + TGF-β1 (magenta curve, tracing F) are significant at 06:30 and all later time points. Successful inhibition of RAC1B was verified by immunoblotting (data not shown).

Table S1: Guide sequences used for knockout of *RAC1* exon 3b

Designation	Sequence (5'→3')
RAC1B CRa	GAGTGTGATAGTTTACCCAC
RAC1B CRb	GCAGGCGTTAAGTTCAACGA
RAC1B CRc	AGCTCGTCCAAGAATCACCG
RAC1B CRd	GTGGGTGCTGCCATGGGAGG

Table S2: Primers used for qPCR

Designation	Sequence (5'→3')	GenBank accession
ALK5-sense	GCGACGGCGTTACAGTGTTTCTGC	NM_004612
ALK5-antisense	ATGGTGAATGACAGTGCGGTTGTGG	NM_004612
β-ACTIN-sense	GACGAGGCCCAGAGCAAGAG	NM_001101
β-ACTIN-antisense	ATCTCCTTCTGCATCCTGTC	NM_001101
RAC1B-sense (exon 3b)	GGGGCAAAGACAAGCCGAT	NM_018890
RAC1B-antisense	CTCGGATCGCTTCGTCAAAC	NM_018890
RAC1-sense	AGGCCATCAAGTGTGTGGTG	NM_018890
RAC1-antisense	AGAACACATCTGTTTGCGGAT	NM_018890
TBP-sense	GCTGGCCCATAGTGATCTTT	M55654.1
TBP-antisense	CTTCACACGCCAAGAAACAG	M55654.1
TBP-sense	AACAACAGCCTGCCACCTTA	M55654.1 (Figure S1)
TBP-antisense	GCCATAAGGCATCATTGGAC	M55654.1 (Figure S1)