

A Lentiviral CXCR4 Overexpression and Knockdown Model in Colorectal Cancer Cell Lines Reveals Plerixafor-Dependent Suppression of SDF-1 α -Induced Migration and Invasion

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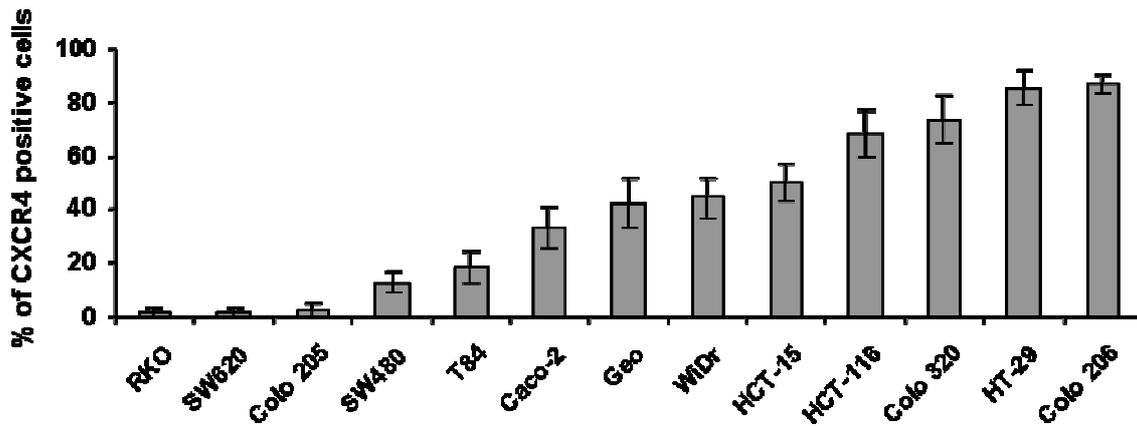
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Supplementary results

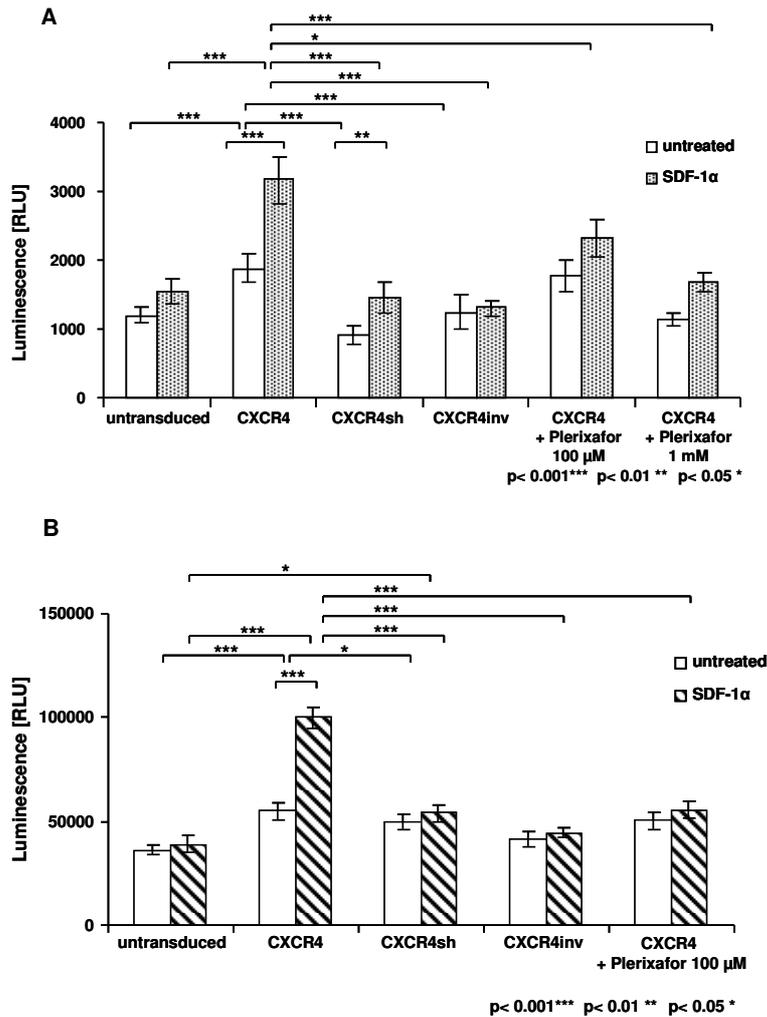
Endogenous CXCR4 expression in different CRC cell lines

In order to establish CXCR4-overexpressing cell lines we initially screened 13 CRC cell lines for cell surface expression of CXCR4 protein using flow cytometry (Supplementary Figure S1). The highest amounts of CXCR4 protein were detected in Colo 206 (87 \pm 3.5% CXCR4-positive cells) and HT-29 cells (85 \pm 6.5%), whereas only a small fraction of RKO (2 \pm 1.5%) SW620 (2 \pm 1.5%) and SW480 cells (13 \pm 4.2%) expressed CXCR4. The latter were chosen for implementing lentiviral gene delivery methods to establish a CXCR4 overexpression model system, especially both SW cell lines were of interest due to their common origin from a patient with colorectal adenocarcinoma (see Ref. 26). Additionally, FACS data together with experienced invasiveness in Matrigel assays supported the selection of RKO cells for further approaches. All 13 cell lines were also checked for SDF-1 α expression levels. No SDF-1 α mRNA was detected by quantitative real-time PCR (data not shown).

Supplementary figures



Supplementary Figure S1: Determination of the amount of CXCR4-positive cells for all 13 CRC cell lines. Cells were stained with anti-human CXCR4-antibody (clone 12G5) and the amount of CXCR4 positive cells was quantified by FACS. Results indicate the mean \pm standard deviation in three independent measurements.



Supplementary Figure S2: Invasion of CXCR4-overexpressing cells. Either SW620 (A) or RKO cells (B) were placed in the upper chamber of a transwell migration flask which had been previously coated with matrigel. Cells were untransduced, or transduced either with HR'SIN-CXCR4-IRES-EGFP (CXCR4) or HR'SIN-CXCR4inv-IRES-EGFP (CXCR4inv). During 24 h, cells invaded through a membrane and the Matrigel towards growth medium, or a chemo-attracting SDF-1 α gradient compounded with growth medium in the lower chamber. Overexpression on a CXCR4-specific shRNA (CXCR4sh) or pretreatment with 100 μ M or 1 mM Plerixafor regulated invasion of CXCR4-overexpressing cells as shown. Results are presented as the mean \pm standard error of luminescence signal derived Relative Light Units (RLU) for up to seven (A), or three (B) independent measurements. See text for exact p-values.