Irinotecan Upregulates Fibroblast Growth Factor Receptor 3 Expression in Colorectal Cancer Cells, Which Mitigates Irinotecan-Induced Apoptosis

Zeynep N. Erdem, Stefanie Schwarz, Daniel Drev, Christine Heinzle, Andrea Reti, Petra Heffeter, Xenia Hudec, Klaus Holzmann, Bettina Grasl-Kraupp, Walter Berger, Michael Grusch, Brigitte Marian

Medical University of Vienna, Department of Medicine 1, Institute of Cancer Research and Comprehensive Cancer Center Vienna

Supplementary Tables and Figures

1. Materials

Quantitative RT-PCR was performed using the Taqman assay kits listed in supplementary table 1.

Supplementary table 1: Taqman gene expression assays used for qRT-PCR

Gene	Catalog no
FGFR1	Hs00915137_m1
FGFR2	Hs01552926_m1
FGFR3-IIIb	Hs01005396_m1
FGFR3-IIIc	Hs00997397_m1
FGFR4	Hs00242558_m1
GAPDH	Hs00266705_g1
FGF8	Hs00171832_m1
FGF9	Hs00181829_m1
FGF18	Hs00818572_m1

2. Supporting Data

2.1. IRI-response in CRC cell line models

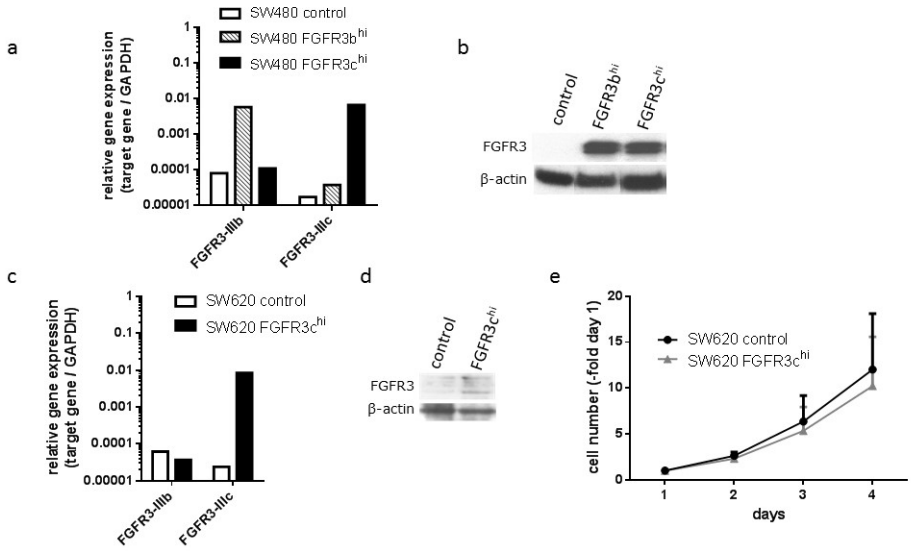
Supplementary Table 2: Irinotecan response and FGFR3 expression in CRC cell lines

cell line	IC_{50}		FGFR3-IIIb		FGFR3-IIIc	
	µM	CI95%	rel. to GAPDH	SD	rel. to GAPDH	SD
SW620	1.3	1.1 - 1.39	6.26	2.76	0.12	0.04
SW480	11.1	9.8-12.5	2.21	0.28	1.88	0.70
HCT116	9.2	8.6-9.7	6.49	4.45	0.40	0.07
Caco2	25.1	22.0-28.5	11.45	3.8	15.55	2.61

The table lists IC_{50} concentrations as well as qRT-PCR results depicted in figure1 of the main text.

2.2. Characteristics of FGFR3-overexpressing clone pools

Supplementary Figure 1: FGFR3-overexpressing CRC cell models:



SW480 FGFR3^{hi} cells were available from an earlier study [1]. SW620 FGFR3^{hi} cells were produced by lipofection and selection of stably expressing clone pools. Expression of the trans-gene was determined by qRT-PCR and was increased >100-fold on the RNA-level (a, c). Receptor protein analysed by Western blot and was clearly detectable in the FGFR3^{hi} cells (b, d). Growth was determined using MTT or SRB assays and was not significantly altered by the transgene (e and Sonvilla et al. 2010).

2.3. Impact of the FGFR-inhibitor PD173074

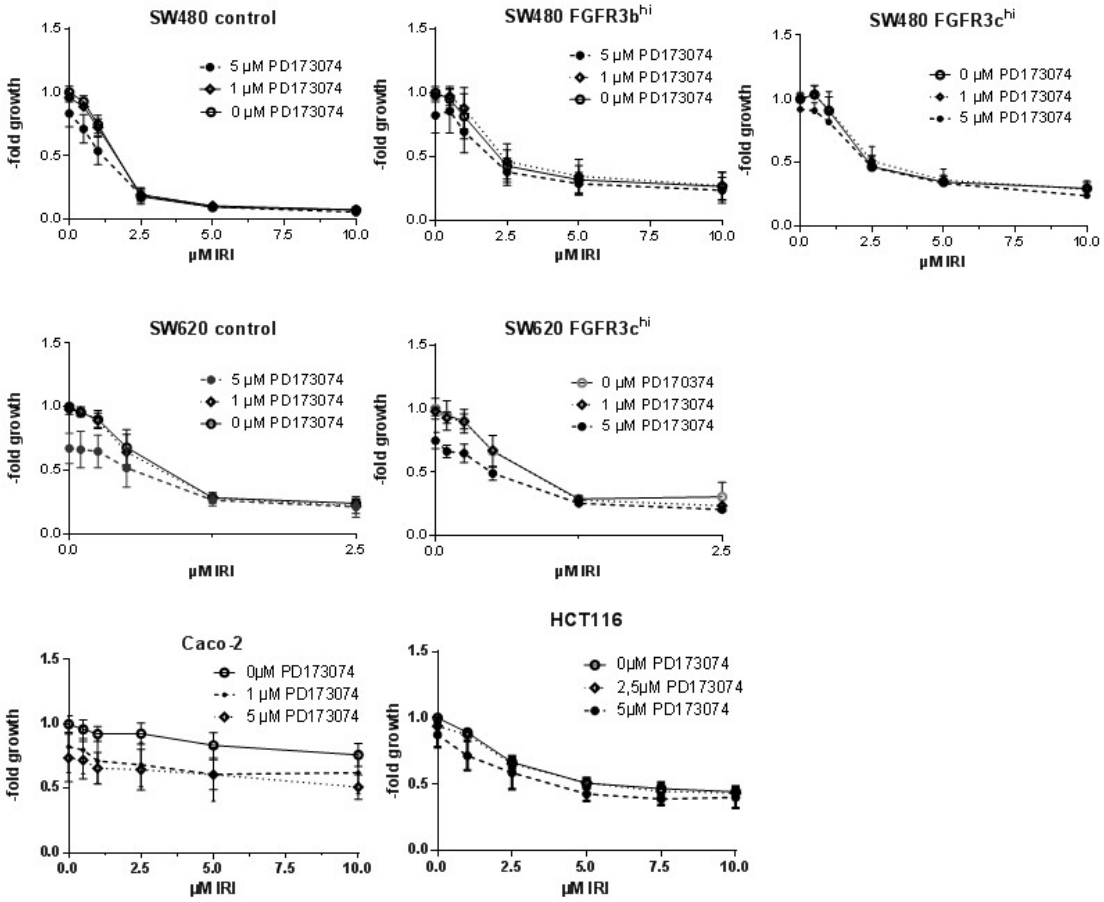
Supplementary Table 3: Sensitivity of CRC cell lines to PD173074

PD173074 is a tyrosine kinase inhibitor specific for FGFRs and was used to block FGFR signaling. It showed cytotoxic activity against all cell lines used in this study. Cells were exposed to the inhibitor for 72 hours and IC_{50} concentrations were calculated by non-linear regression using GraphPad Prism software.

Cell Line	IC_{50} PD173074 (µM)	95% CI
SW480		
control	15.1	14.6-15.6
FGFR3b^{hi}	15.1	14.6-15.5
FGFR3c^{hi}	14.9	14.5-15.3
HCT116	12.4	11.9-12.9
Caco2	9.1	8.5-9.6

Supplementary Figure 3: Combination treatment with IRI and PD173074

For combined treatment with IRI and PD 173074, cultures were exposed to increasing concentrations of either compound or both for 72 hours. Then viability was determined and dose response curves were constructed. The data depicted in the figure were used for the computation of combination indices (CI) using CompuSyn software. CI are summarised in the main text in table 2.



3. Reference

Sonvilla, G., et al., *Fibroblast growth factor receptor 3-IIIc mediates colorectal cancer growth and migration.* Br J Cancer, 2010. **102**(7): p. 1145-1156.