In vitro evaluation of Rigosertib antitumoral and radiosensitizing effects against human cholangiocarcinoma cells

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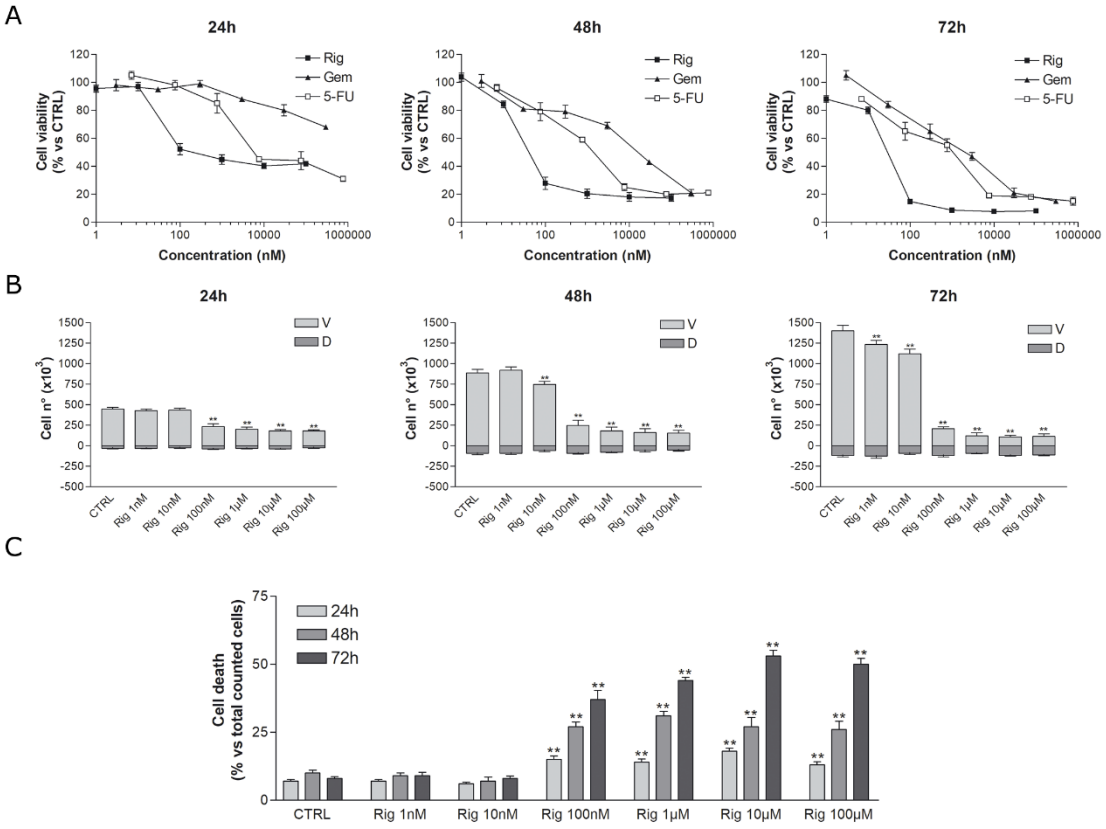
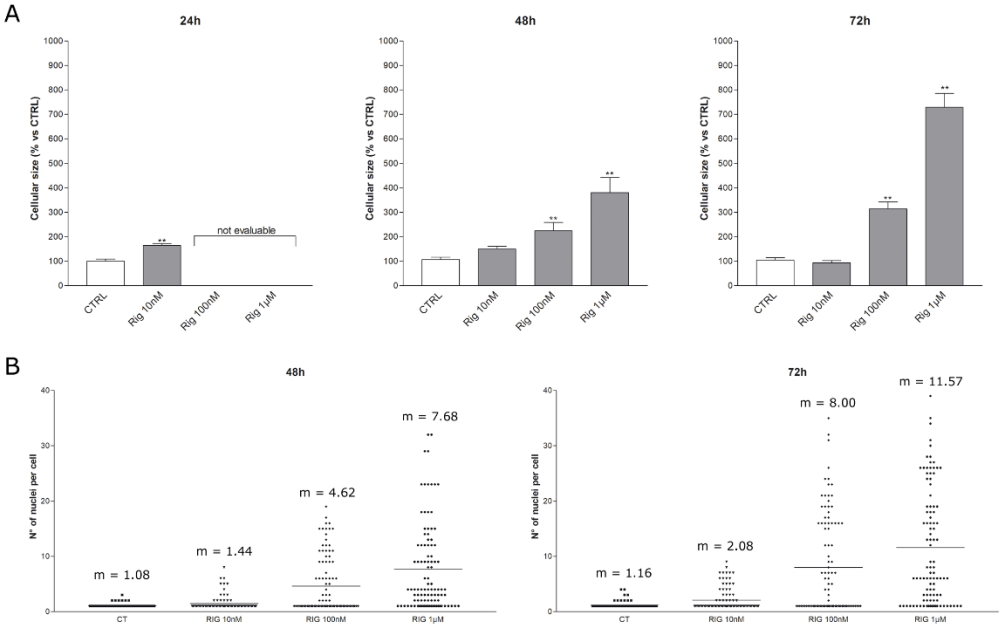
Supplementary Table S1. Percentage of counted EGI-1 death cells after Gem and 5-FU treatments in Trypan blue assay (* p <0.05, ** p <0.01 vs CTRL).

[Gem]	24h	48h	72h
CTRL	5.3 ± 1.2	7.5 ± 1.8	11.9 ± 1.2
3nM	5.4 ± 1.1	8.2 ± 1.7	11.9 ± 1.8
30nM	5.0 ± 1.0	10.8 ± 3.0	12.9 ± 1.8
300nM	5.8 ± 1.2	12.8 ± 3.3	11.7 ± 1.7
3μM	6.4 ± 1.0	19.5 ± 2.4 **	23.4 ± 2.4 **
30μM	7.1 ± 1.1	29.9 ± 4.5 **	48.0 ± 9.2 **
300μM	11.8 ± 2.1 **	50.9 ± 5.9 **	70.8 ± 13.2 **

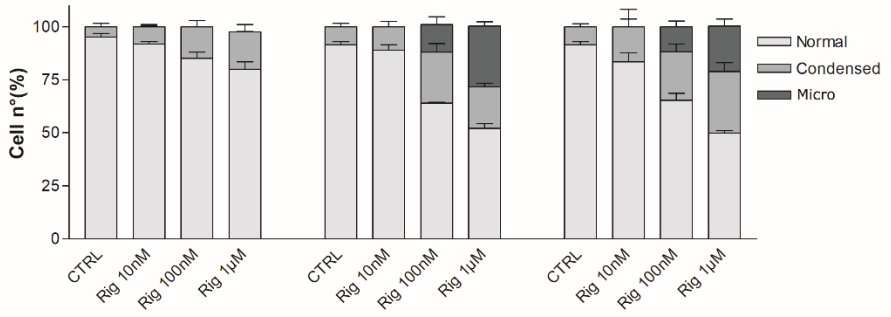
[5-FU]	24h	48h	72h
7nM	6.2 ± 1.2	9.5 ± 1.5	14.9 ± 2.1
70nM	7.8 ± 1.0	11.5 ± 2.1	20.8 ± 3.1
700nM	9.8 ± 2.6	15.7 ± 3.3	21.2 ± 2.9
7μM	12.5 ± 2.7 *	24.2 ± 5.8 **	48.8 ± 6.7 **
70μM	16.7 ± 3.1 **	27.0 ± 4.8 **	45.4 ± 18.7 **
700μM	21.8 ± 4.4 **	29.6 ± 6.2 **	48.12 ± 10.6 **



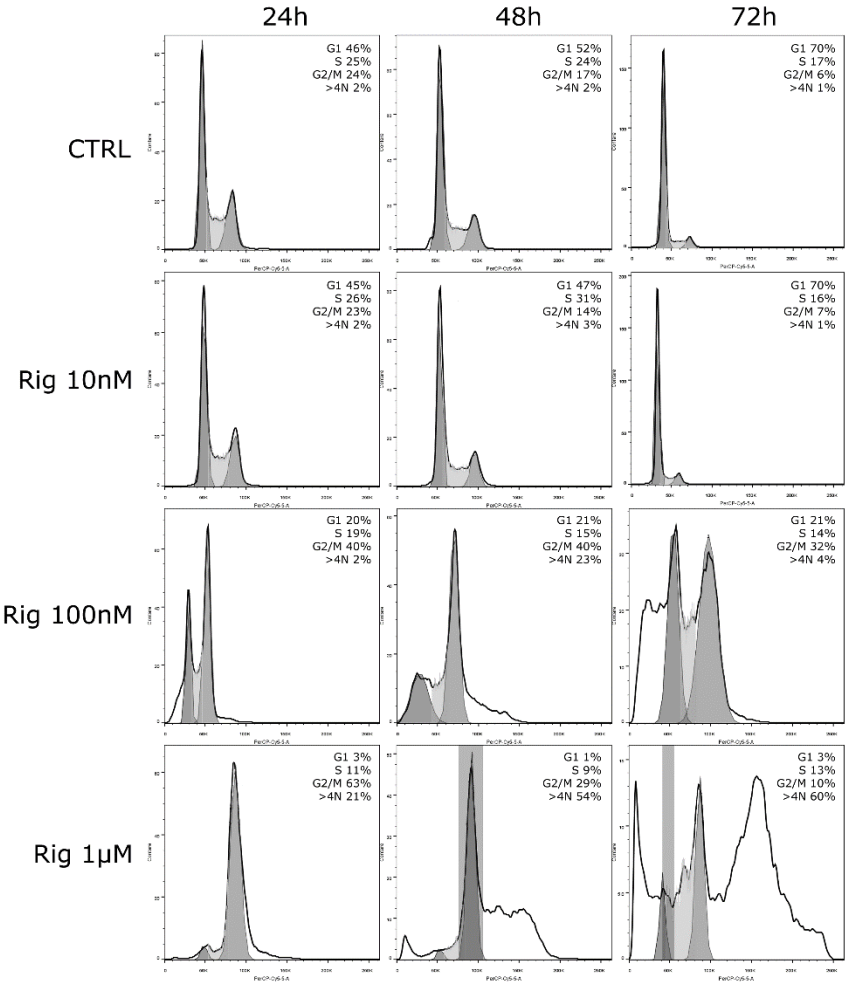
Supplementary Figure S1. . Trypan blue vital count of TFK-1 cells treated with Rig, Gem or 5-FU. (A) Percentage of viable cells after treatment with different concentrations of Rig (1nM – 100µM), Gem (3nM – 300µM) and 5-FU (7nM – 700µM). **(B)** Number of viable (V) and dead (D) cells treated with increasing concentrations of Rig (1nM – 100µM) for 24, 48 and 72h. **(C)** Percentage of counted TFK-1 death cells after Rig treatment. The percentage is calculated on the total number of counted cells. Data are presented as the mean ± SD of at least three independent experiments (** p <0.01 vs CTRL).



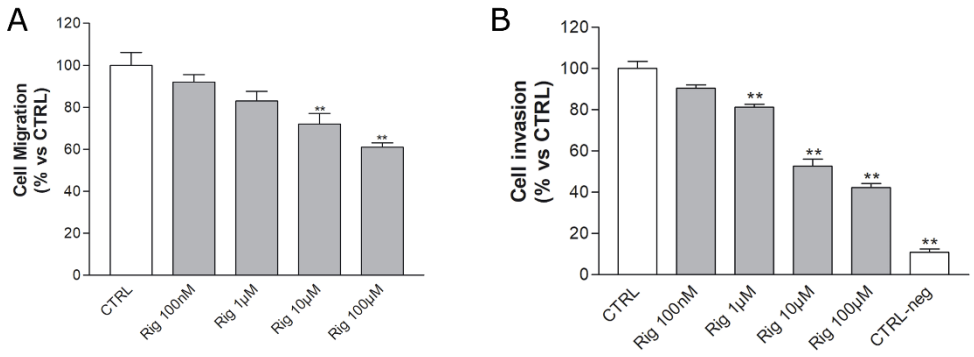
Supplementary Figure S2. Cell size and nuclei number of TFK-1 cells treated with Rig. (**A**) Graphs represent the mean ± SD size of cells treated with increasing concentrations of Rig (1nM – 100µM) for 24, 48 and 72h. (**B**) Graphs represent the number of nuclei/micro-nuclei counted in each TFK-1 cell (at least 100 cells counted for each condition) treated with increasing concentration of Rig (10nM – 1µM). Horizontal black line and the number on the top represents the mean number of nuclei/micro-nuclei. (p <0.01 vs CTRL).



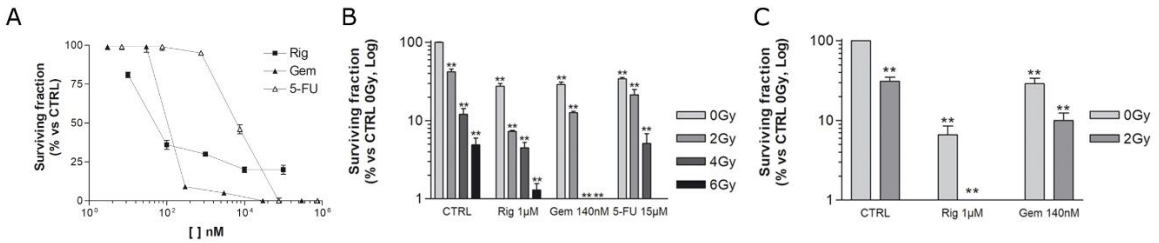
Supplementary Figure S3. GIEMSA staining of TFK-1 cells treated with Rig. Graph represent the mean ± SD percentage of cells with normal nuclei (normal), condensed nuclei (condensed) or micro-nucleated (micro), after treatment with increasing concentrations of Rig (10nM – 1µM) for 24, 48 and 72h. Data are presented as the mean ± SD of three independent experiments. (* p<0.05, ** p <0.01 vs CTRL).



Supplementary Figure S4. Cell cycle analysis of TFK-1 cells treated with Rig. Representative histograms of the distribution of TFK-1 cells in the different phases of cell cycle after treatment with Rig 10nM, 100nM and 1µM, for 24, 48 and 72h.



Supplementary Figure S5. TFK-1 cell migration and invasion. (A) Scratch wound healing assay of TFK-1 cells treated with increasing concentrations of Rig. The graph represent the mean ± SD percentage of the area of cells that were able to close the scratch after treatment with increasing concentration of Rig compared to corresponding untreated control cells. **(B)** Boyden chamber assay of TFK-1 cells treated with increasing concentrations of Rig. Graph represent the percentage of cells that are able to pass through the membrane. CTRL and CTRL-neg represents cells without any treatment that passed through the membrane respectively in presence or in absence of serum in low chamber. Graphs are the mean ± SD of three independent experiments. (* p <0.05, ** p <0.01 vs CTRL).



Supplementary Figure S6. Clonogenic assay of TFK-1 cells and Rig. (A) Clonogenic assay of TFK-1 cells treated with increasing concentrations of Rig (1nM-100µM), Gem (3nM-300µM) and 5-FU (7nM-700µM) without radiations. **(B)** Clonogenic assay of TFK-1 cells treated with Rig 1µM, Gem 140nM and 5-FU 15µM for 24h, and irradiated with increasing doses (0-6Gy). **(D)** Clonogenic assay of TFK-1 cells treated with Rig 1µM and Gem 140nM for 48h, and irradiated with increasing doses (0-6Gy). (** p<0.01 vs CTRL).