Targeted cancer therapy based on blocking the expression of genes and small doses of oxaliplatin.

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Introduction. Since majority of cancers eventually develop resistance to the prescribed chemotherapy, so doctors tend to perform combination treatment by means of targeted drugs. The major drawback remains significant side effects and resistance to standard therapy. Our **purpose** was to identify potential biological targets associated with the development of drug resistance and to develop a specific method of suppressing the viability of colon cancer cells exposed to low doses of standard chemotherapy.

Materials: colon cancer cells HCT-116.

Methods: 1.) Network analysis and the selection of genes, that potentially associated with oxaliplatin resistance. 2.) Evaluation of the oxaliplatin responsive genes by means of oxaliplatin treatment (at low dozes) of colon cancer cells, followed measurement of gene's expression using Reverse-Transcription and Real-Time PCR. 3.) siRNA design and transfection of cells treated with low dozes of oxaliplatin in order to silence the selected candidate gene markers of oxaliplatin resistance. 4.) Cell viability and apoptosis measurement

Results: 15 genes were selected for this study according to their involvement in colon cancer and oxaliplatin resistance. The following online resources were used for this selection:
https://www.oncomine.org/resource
http://biogps.org
http://mirob.interactome.ru/

We found that at low concentrations of oxaliplatin (5 and 10μM), the survival rates of colon cancer cells HCT-116 do not differ from the untreated cells (UC) during the period of 48 hours of incubation (Figure 1), but the levels of the observed expression of genes were different (Figure 2).

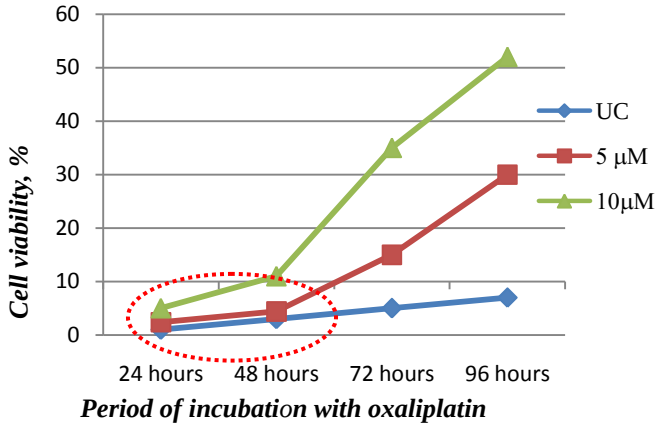


Figure 1. Cell viability under oxaliplatin exposure

Bcl2				
Bcl-xL				
cIAP1				
cIAP2				
XIAP				
Survivin				
LIVIN				
FLIP				
GRP78				
TRAP1				
cMYC				
Gstp1				
Gstm2				
Gstm4				
ERCC1				
Incubation period	**24 hours**	**48 hours**	**24 hours**	**48 hours**
Doze	**5 μM oxaliplatin**		**10 μM oxaliplatin**	

Left –to right: downregulation

Left –to right: upregulation

Figure 2. Profile of gene expression panel under time and doze depended exposure

Notes: *HCT-116 cells were treated with 5 or 10 μM of oxaliplatin. Fold changes were determined by ΔΔCt relative quantification method. Samples are the genes from the cells that were treated with oxaliplatin, and reference are the genes from the untreated cells. Gene GAPDH was used as the endogenous control.*

Small interfering RNA(siRNA) design

siRNA oligonucleotides that knock down messenger (m)RNAs of the genes *cIAP2* and *LIVIN* and non-targeting (scrambled) siRNAs were designed using the program BLOCK-iT™ RNAi Designer (Life Technologies/ThermoFisher Scientific).

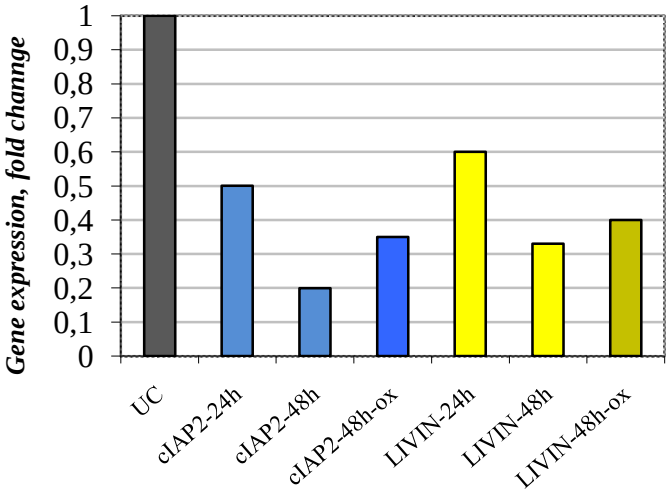
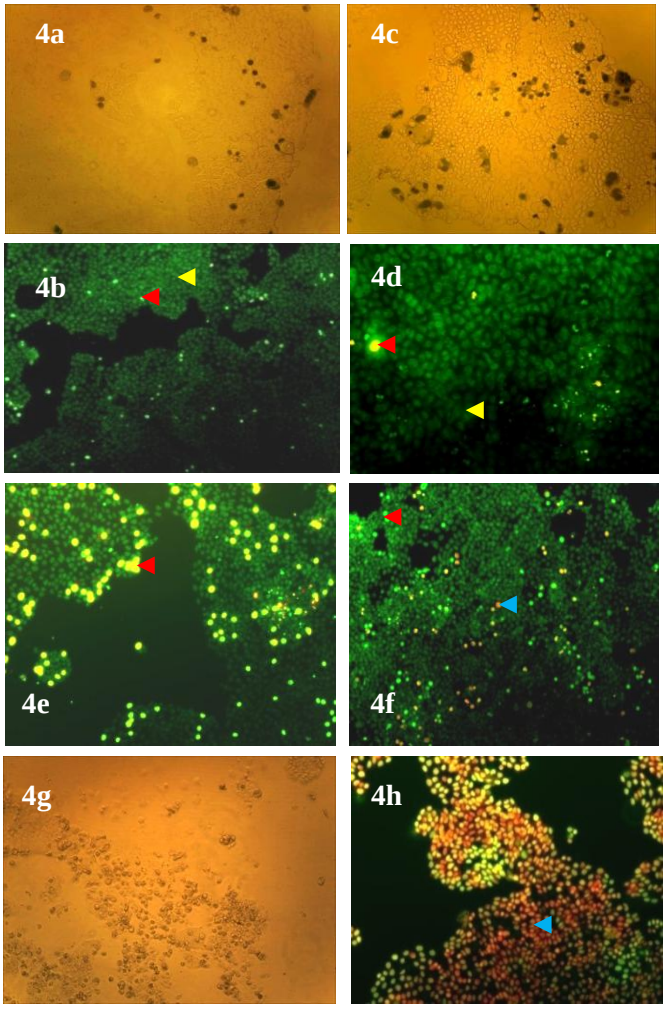


Figure 3. **Anti-*cIAP2* and anti-*LIVIN* siRNAs downregulate *cIAP2* and *LIVIN* in HCT-116 cells**.
Notes: *Cells were transfected with anti-cIAP2 or LIVIN siRNAand incubated for 24 and 48 hours ox-free and with ox (10 μM). Expression levels were analyzed using real time-PCR.*
Abbreviations: *h, hours; ox, oxaliplatin; UC, untreated cells.*

The evaluation of siRNAsilencing

For *cIAP2*, the maximum rate of silencing was obtained in 48 hours by means of 15 nM of siRNA and was as much as 80% (P <0.024) without oxaliplatin and 65% when 10 μM of oxaliplatin was added (P <0.01). Similar results were observed for LIVIN (Figure 3).

HCT-116 cells containing 10 μM of oxaliplatin were transfected with 15 nM of either anti-*cIAP2* or *LIVIN* siRNAs or both (cocktail). After 48 hours of incubation, the cells were stained with either Hoechst 33342/propidium iodide (Figure 4b-f and 4h) or Trypan blue (Figure 4a, 4c and 4g). Apoptotic cells were examined under a fluorescent microscope with ten fields of view per well (10×/20× magnification). Silencing of each gene resulted in a two-fold increase in sensitivity to oxaliplatin (P<0.01) (Figure 4i) compared to the cells treated with only oxaliplatin. A significant rate of apoptosis (P <0.005) was achieved by means of combination of *cIAP2* and *LIVIN* (Figures 4i and 4g,4h).



4i

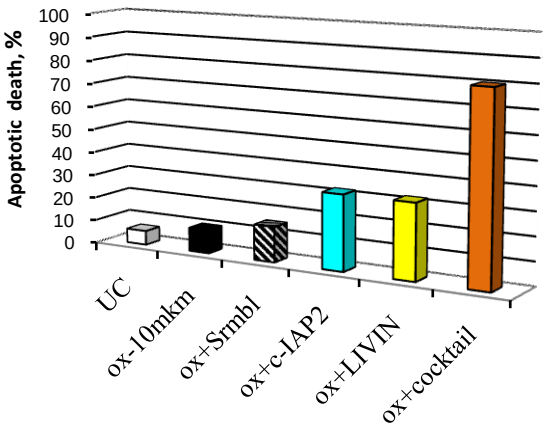
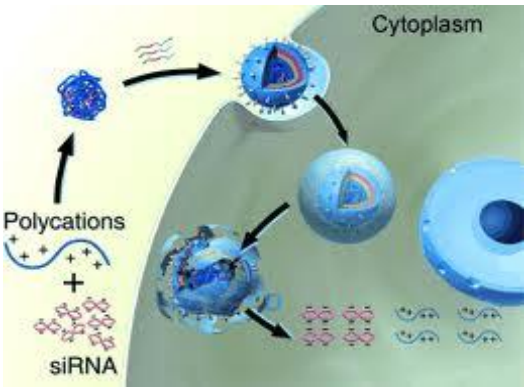


Figure 4. The elevated response to oxaliplatin of HCT-116 cells that were treated with anti-LIVIN and anti-cIAP-2 siRNAs.
Notes: *4a, 4b – untreated cells (UC);4c and 4d – cells treated with 10μM of oxaliplatin;4e – cells treated with 10μM of oxaliplatin and anti-cIAP2 siRNA; 4f - cells treated with 10μM of oxaliplatin and anti-LIVIN siRNA; 4g and 4h - cells treated with 10μM of oxaliplatin and both siRNAs (cocktail). Red arrows indicate apoptotic cells, yellow – live cells, blue – necrotic cells. 4i – comparative evaluation of the apoptotic cell death.*

Conclusion

The siRNA cocktail appears to be an effective tool with which to inhibit several targets that are essential for cancer cell proliferation or survival activity. Over the last 3 years, a number of authors have published results showing a successful cooperative effect in other types of cancers by employing two or three different siRNAs, including *cIAP1*, *cIAP2*, and *XIAP* for prostate cancer or *XIAP*, *LIVIN*, and *Survivin* for bladder cancer. Together, these findings provide new insight into approaches for the prevention of drug resistance in cancer cells.

Further plans: drug design, delivery to the cancer cell coated of nanoparticles



Published articles related to this topic:

Bavykin A.S., Korotaeva AA., Syrtsev, Karpukhin A. V., Tulyandin S.A. The development the effectiveness of chemotherapy by means of small interfering RNAs. *J. Malignant Tumors 2012, T2, N2, p.111-116.*

Bavykin AS, Korotaeva AA, Poyarkov SV, Syrtsev AV, Tjulandin SA, Karpukhin AV.
Double siRNA-targeting of *cIAP2* and *LIVIN* results in synergetic sensitization of HCT-116 cells to oxaliplatin treatment. *OncoTargets and Therapy, 2013, Volume 6, p: 1333 – 1340.*

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