EV-encapsulated small molecule inhibitor decreases viability in cancer cell lines

Eleana Hatzidaki[a], Ioanna Vlachou[a], Maria Papadimitriou[a], Ioannis Papasotiriou[a]

[a] *Research Genetic Cancer Centre S.A., Industrial Area of Florina, Florina, Greece*

ANNUAL MEETING ISEV2018 2-6 May Barcelona, Spain ISEV2018.ORG

Poster #: PS02 02

INTRODUCTION

Here in Research Genetic Cancer Centre (RGCC) we are in the process of developing a novel small molecule ERK inhibitor. We have currently synthesized an intermediate molecule -RGCC169- which needed to be tested in order to confirm we are using the appropriate tools. The limited solubility that this compound exhibits makes it difficult to enter the cell membrane and exert its effects. Therefore, a delivery system had to be devised in order to increase intracellular uptake efficiency.

MATERIALS & METHODS

EVs were isolated from human serum by PEG precipitation. EV intracellular fate was determined by fluorescence microscopy using anti-CD63 Ab during various time points. RGCC169 was EV encapsulated and loading was determined by HPLC using both AcN and MeOH. RGCC169 cell sensitivity was determined using both a Her2 negative, PIK3CA mutated (MCF7) and a Her2 positive, PIK3CA/KRas mutated (HCT116) cell line. EV-encapsulated RGCC169 toxicity was evaluated by MTT viability assay on MCF7 cells.

RESULTS

We have successfully loaded our compound into EVs as evident from HPLC experiments. AcN vs MeOH mobile phases give different loading efficiencies (*Table 1*). EVs are delivered intracellularly by endocytosis within 30mins (*Figure 1*). Sensitivity to RGCC169 was greater in MCF7 cells (PIK3CA mutated) as shown in *Figure 2*. Encapsulated RGCC169 was shown to have increased cytotoxicity over RGCC169 alone (*Figure 3*).

	Average loading efficiency	SEM	No of experiments
AcN	28	0.6	9
MeOH	84	2.7	2

Table 1: Different loaded efficiencies using either methanol or acetonitrile as a mobile phase in HPLC

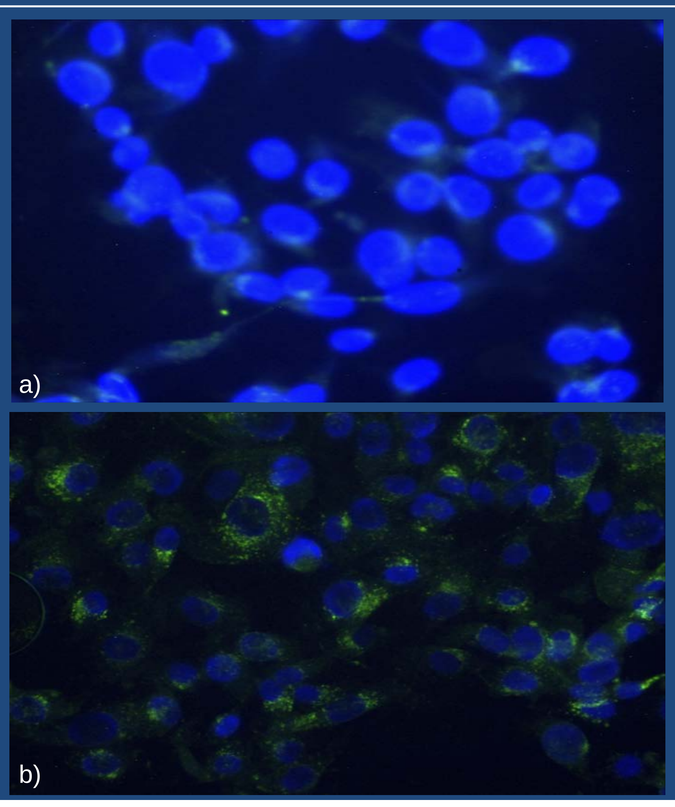

Figure 1: EV cellular uptake at 30mins as evident under fluorescent microscope. MCF7 cells exhibit very little CD63 expression (a). CD63 fluorescence in EV-CD63 incubated MCF7 is extensive (b).

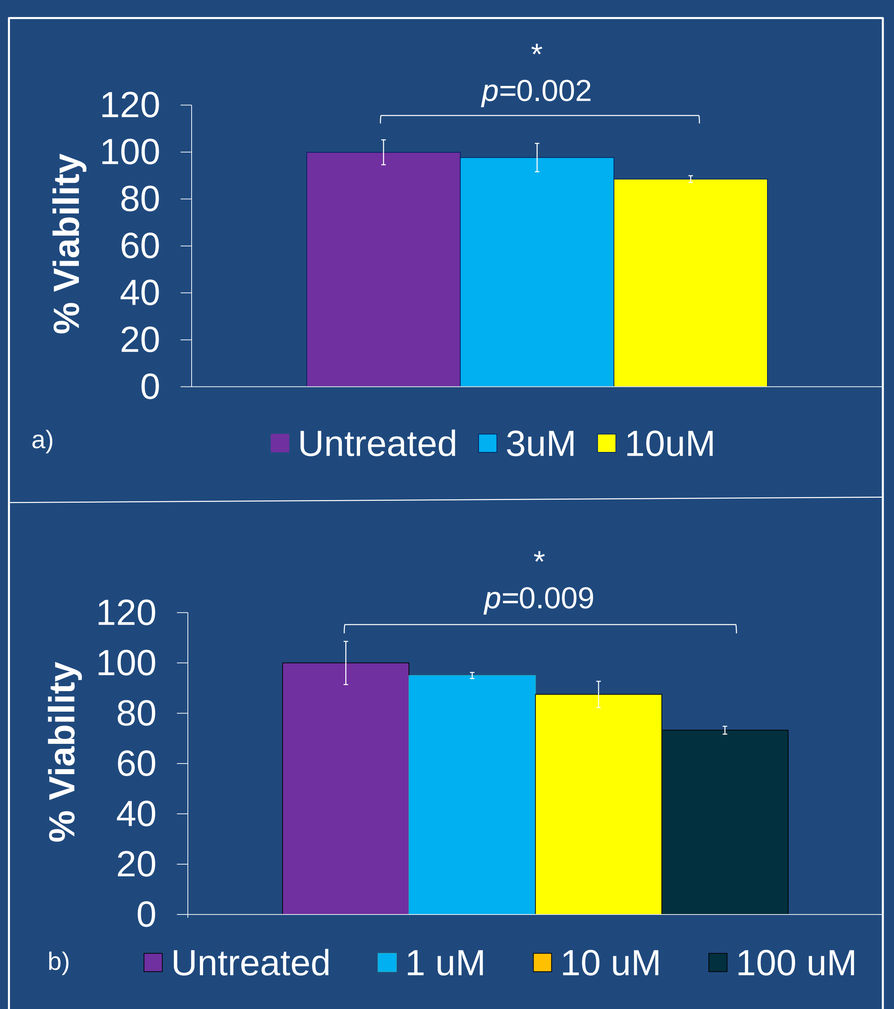

Figure 2. The effect of RGCC169 on MCF7 (a) and HCT116 (b) cell viability. For MCF7 3uM and 10uM RGCC169 were used. For HCT116, 1uM, 10uM and 100uM RGCC169 were used

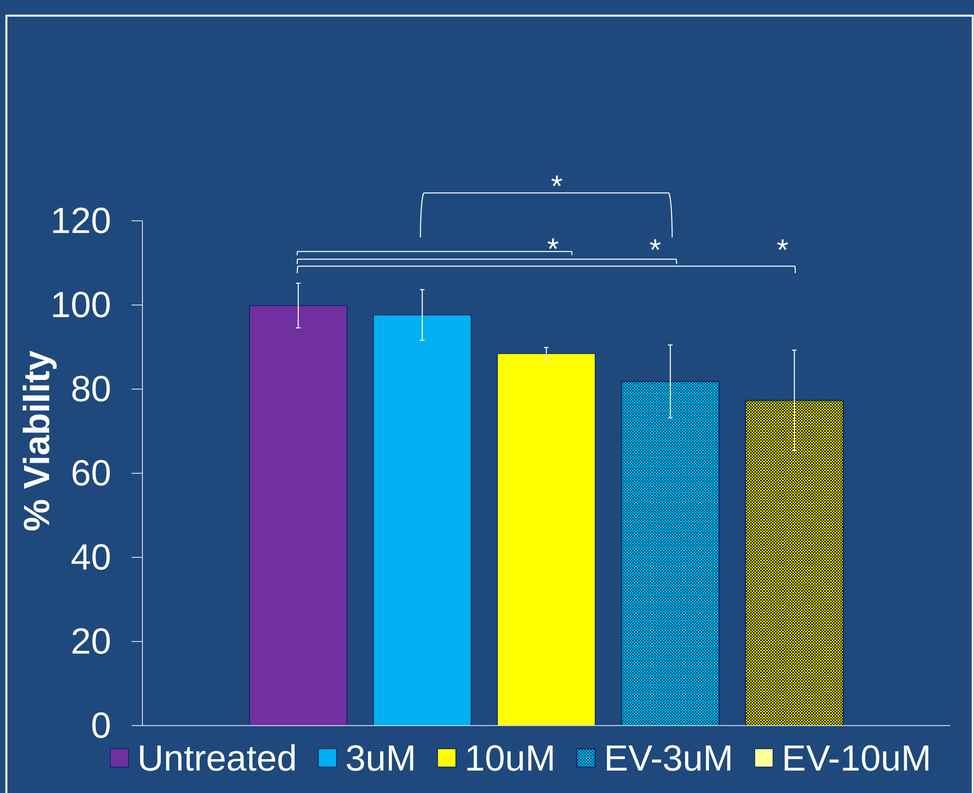

Figure 3: The effect of 3uM, 10uM RGCC169 and 3uM, 10uM EV-encapsulated RGCC169 on MCF7 cell viability. (Untreated *vs* 10uM p=0.002, Untreated *vs* EV-3uM p=0.0004, Untreated *vs* EV-10uM p=0.0009, 3uM *vs* EV-3uM p=0.002

CONCLUSION

MeOH gives higher encapsulation efficiency compared to AcN. This could either be due to the greater ability of MeOH to break apart EV pellets, or due to great variability of loading. EVs are delivered by endocytosis. Her2 positive, PIK3CA/KRas mutated cell lines are less sensitive to RGCC169 possibly due to the higher levels of activated ERK. EV encapsulation increased significantly cell sensitivity to RGCC169.

The above findings confirm that we have successfully devised a delivery system for our novel molecule's intracellular transport and that we are indeed using the appropriate synthesis methods for the achievement of our final goal; that is the synthesis of a novel cytotoxic drug. Future perspectives include elucidating RGCC169 mechanism of action.

SELECTED REFERENCES

- Laulagnier K1, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, Kobayashi T, Salles JP, Perret B, Bonnerot C, Record M. (2004). Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J. 380(Pt 1):161
- Witwer K., Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Notle-'t Hoen EN, Piper MG, Sarada S., Skog J, Thery C, Wauben MH, Hochberg F. (2013)Standardization of sample collection, isolation and analysis methods in extracellular vesicel research. Journal of extracellular vesicles, 2:1, 20360
- Théry C1, Ostrowski M, Segura E. (2009). Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 9(8):581
- Nan Der Pol E., Boing AN, Harrison P, Sturk A, Nieuwland R. (2012). Classification, Functions and Clinical Relevance of Extracellular Vesicles, Pharmacological Reviews, 64(3): 676-705.