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Development of an extracellular vesicle-associated transcriptomic biomarker signature for vandetanib treatment response of anaplastic thyroid cancer cells

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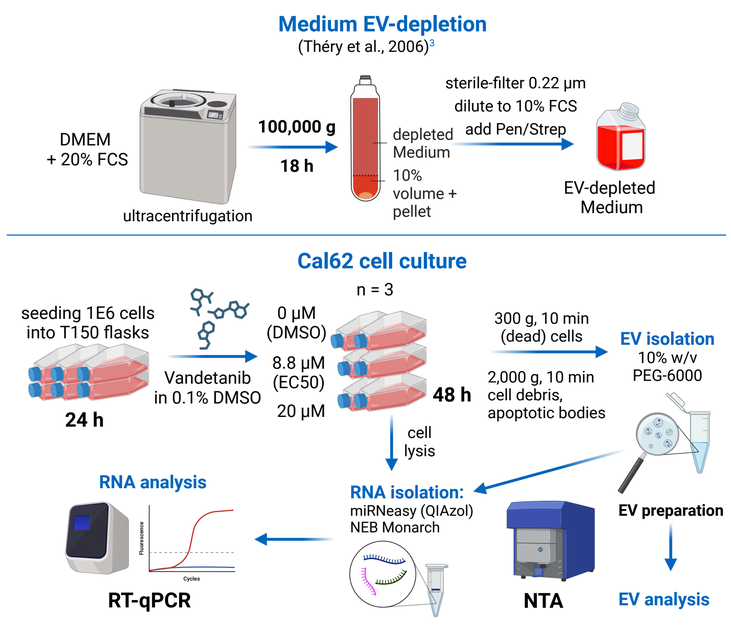
INTRODUCTION

Anaplastic thyroid cancer (ATC), one of the malignancies included in the REPO4EU drug repurposing project, is a relatively rare but highly aggressive form of thyroid carcinoma (TC), with high metastatic tendency and a lack of targeted therapy options[1]. We found sensitivity of the ATC cell line Cal62 to the receptor tyrosine kinase (RTK) inhibitor vandetanib. To assess response to a drug in clinical practice, imaging techniques are usually employed, taking time to identify non-responders, in which the tumor can grow and metastasize further. Since tumors are known to release a high amount of cell-free RNA (cfRNA), which can be found in patient blood samples and is protected from degradation by association to extracellular vesicles (EVs)[2], a liquid biopsy-based approach might provide earlier information about the therapeutic success.

AIM

We aimed to identify an *in vitro* candidate EV-associated cfRNA biomarker signature for the response of Cal62 cells to vandetanib. As a first step, we used RT-qPCR assays to assess the transcriptomic changes introduced in Cal62 cells and their extracellular vesicles upon treatment with vandetanib.

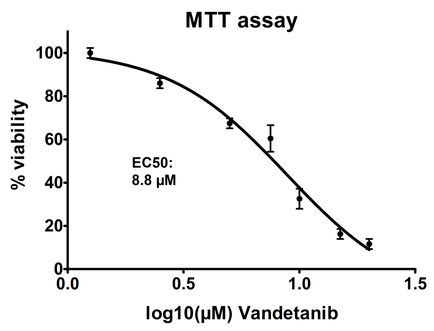
METHODS



Graphical representation of the workflow.

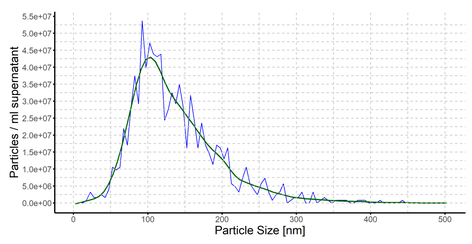
RESULTS

Cell Viability and EC50



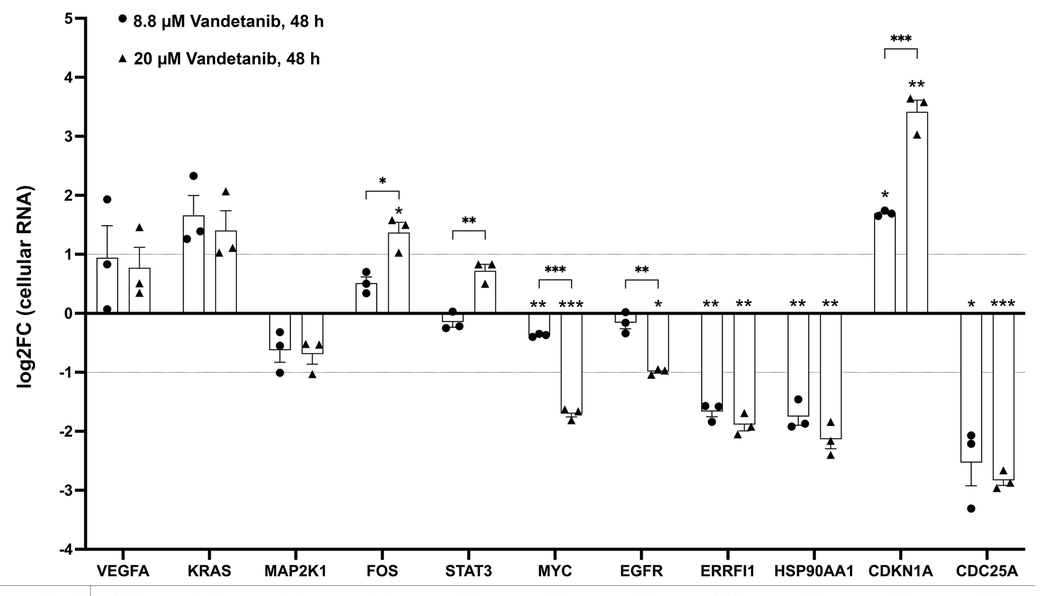
Dose-response curve of Cal62 cells treated with vandetanib, measured using an MTT-based Assay in a 96-well plate (2000 cells/well). Error bars represent the standard error of the mean (SEM). The EC50 value was calculated as 8.8 µM using a four-parameter non-linear model with variable slope in GraphPad Prism.

EV Characterization



Exemplary size profile of Cal62 EVs isolated from the cell culture supernatant (20 µM vandetanib treatment), measured on a Zetaview NTA (Particle Metrix). The size distribution and particle concentration is representative for all samples.

Gene Expression Analysis



		VEGFA	KRAS	MAP2K1	FOS	STAT3	MYC	EGFR	ERRFI1	HSP90AA1	CDKN1A	CDC25A
Fold Change (cellular RNA)	8.8 µM	+ 2.35x	+ 3.53x	- 1.47x	+ 1.49x	- 1.06x	- 1.25x	- 1.07x	- 3.05x	- 3.22x	+ 3.36x	- 5.18x
	20 µM	+ 1.89x	+ 2.91x	- 1.56x	+ 2.68x	+ 1.70x	- 3.17x	- 1.92x	- 3.58x	- 4.22x	+ 11.22x	- 6.89x
Fold Change (EV-/cfRNA)	8.8 µM						↓			-5.14x	-1.45x	
	20 µM						↓			-2.97x	-3.56x	

Vandetanib-induced differential expression of genes in Cal62 cells and their EVs after 48 h (n=3). The relative gene expression was measured using RT-qPCR, and the results for cellular RNA are plotted as log2 fold change (log2FC, both mean values and single data points). Additionally, the mean fold change (FC) in the cellular RNA is provided for each gene in the table below. For three of the target genes, the FC in the EV-associated cell-free RNA (cfRNA) is also provided. Detection of the remaining target genes was not possible on cfRNA level due to their low expression levels. All data was normalized to the three non-regulated reference genes GAPDH, UBC and ARF1. This combination of reference genes was identified as the most stable choice among all reference gene candidates using the algorithms geNorm[4], NormFinder[5] and BestKeeper[6], since their mean expression showed the smallest variation in the given experimental setting. All relative expression values were corrected for the efficiency of the corresponding RT-qPCR assays[7]. Error bars represent the standard error of the mean (SEM). A two-sided t-test was used to determine significance. Asterisks represent p-values <0.05 (*), <0.01 (**) and <0.001 (***).

CONCLUSION

We showed that vandetanib induces significant changes in the expression levels of several transcription factors of the MAPK pathway (**FOS**, **STAT3**, **MYC**) in Cal62 cells. This contributed to dysregulation of genes involved in cell cycle arrest (**CDKN1A**, **CDC25A**), **EGFR** feedback signalling (**ERRFI1**) and cellular stress (**HSP90AA1**). Loss of **HSP90AA1** expression has also been associated with a better outcome in clinical studies[8]. Interestingly, the expression levels of **VEGFA**, the main ligand of VEGFR-2, one of the main targets of vandetanib, as well as the MAPK pathway kinases **KRAS** and **MAP2K1** were not affected significantly by vandetanib treatment. The same trends were also found in the Cal62 EV-RNA. As a next step, we will assess how the transcription of these genes is regulated after different treatment times with vandetanib. Finally, we will investigate the complete transcriptomic change of Cal62 cells in the cellular and cell-free RNA by next-generation sequencing to identify an EV-associated biomarker signature that significantly predicts the response to vandetanib in vitro. Furthermore, we will assess the EV proteome and the proteomic changes introduced by vandetanib in Cal62 EVs. These results may serve as a basis for further clinical studies, making this project an important basis towards assessing therapeutic success of vandetanib treatment for ATC patients in liquid biopsies.

References:

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