# Novel Strategies to Target Mitochondria in Bile Duct Cancers

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## INTRODUCTION

Mitochondria (Mi) are fundamentally implicated in cancer biology, including initiation, growth, metastasis, relapse, and acquired drug resistance. Mi are considered as the target organelles for therapeutic strategies of several cancers including CCA. This project aims to provide a novel approach targeting Mi in CCA, by elucidating at molecular level a novel Mi-related cell death pathway, recently identified by our group, who first showed that genetic alterations in HCCS and COX7B, two components of the mitochondrial respiratory chain, cause apoptosis induced by APAF1-independent CASP9 activation(1).

## AIM

The project aims

to dissect the the novel Mitochondrial Apoptosis Pathway,

to identify all molecular players,

to functional characterize their role in CCA,

to identify novel targeted therapies,

to develop a gene signature exhibiting consistent prognostic power and predictive value as potential biomarker.

## METHOD

- HTS has been performed by using Prestwick Chemical Library (Prestwick).
- The Human siGENOME Druggable-Genome-Library (Dharmacon) has been used in functional experiments.
- CASP9 activity has measured using the ApoAlert CASP9 assay (Clontech).
- Cell viability was monitored by CellTiter-Blue assay (Promega).
- The patient cohort consists of 100 patients retrospectively identified by Dept of Oncology (Univeristy of Modena).
- FFPE-RNA extraction, RNAseq and analyses were performed using protocols available at TIGEM(2).
- Lentiviral-based shRNA and CRISPR/Cas9 systems were utilized to establish stable gene knockdown and knockout model.

## RESULTS

Molecular players of the novel MiPa, which triggers APAF1-independent caspase-9 activation have been identified using a proteomic approach combined to siRNA library screening (Fig.1).
HTS allowed to identify compounds targeting the novel MiPa. Two compounds were found to inhibit tumour growth *in vitro* and their anti-tumour efficacy has been assessed (Fig.3).
Experiments in HuCCT-1, KMCH, CCLP, SW1, EGI, TFK1 cell lines revealed their effect on autophagy inhibition and apoptosis and correlated the MiPa pathway with drug treatment response and drug resistance (Fig. 3 – results showed for selected cell lines).
Whole-transcription sequencing experiments are ongoing to profile the novel MiPa gene signature in cell lines treated and not with standard therapeutic approach, and in a cohort of 100 CCA patients (Fig.2).

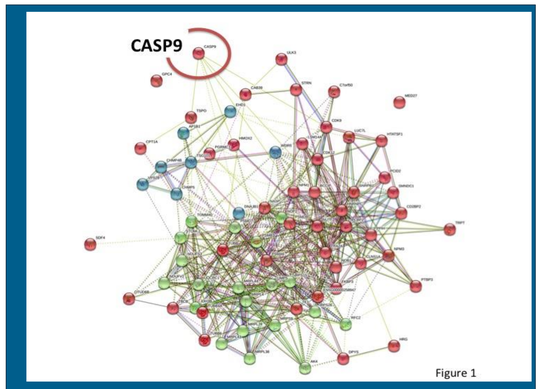
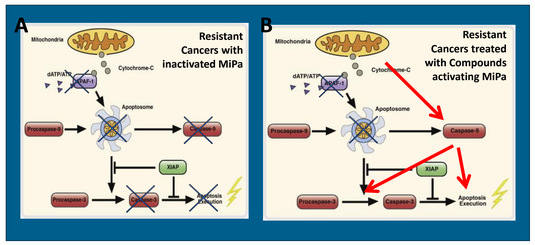



Figure 1

*Figure 1 – String Analysis Interactome Profile of proteins interacting with activated CASP9 in APAF1 negative cells (pvalue<0.05)*



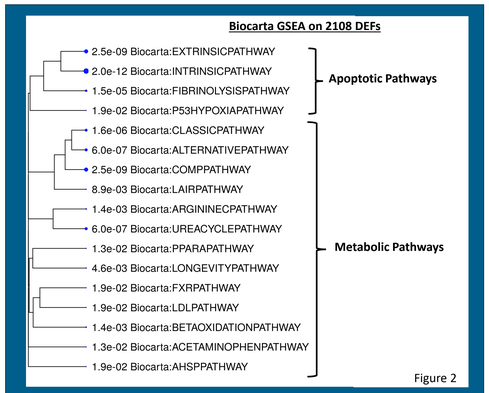




Figure 2

*Figure 2 – Gen Set Enrichment Analysis using Biocarta categorization of differentially expressed genes (DEF) between tumour and non tumours samples in 30 CCA patients. The hierarchical clustering tree showed a significative correelation between 2108 DEFs and Apoptotic and Metabolic pathways (pvalue<0.05; FDR<0.25)*

*Figure 4 – Molecular Model of new therapeutic strategy. Drug-Resistant Cancers frequently are able to evade cell death by inhibiting mitochondrial apoptosis (A). We identified a new therapeutic strategy to activate mitochondrial apoptosis in cancers where cell death machinery is blocked or inhibited (B).*

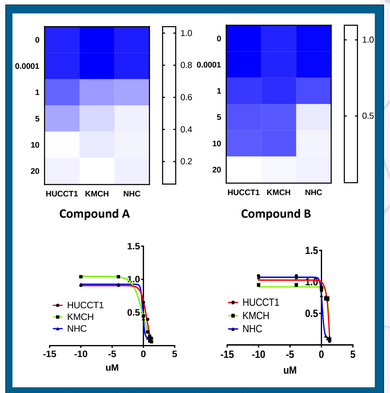



*Figure 3 – High throughput Library Compound screening allowed to identify 2 hit-compounds targeting the novel apoptotic pathway. The 2 hit-compounds inhibit tumor growth both in vitro (Dose-response proliferation assays and GI50 calculation) both in vivo (data not showed).*

## CONCLUSIONS

A novel MiPa has been identified regulating the apoptosis in CCA. Implication of the novel MiPa in drug resistance and sensitivity to classical therapeutic treatment has been assessed (Fig.4).
Two compounds were identified to target the novel MiPa. Whole-transcriptome analysis are ongoing to evaluate the clinical value of the MiPa gene signature in CCA patients.

## REFERENCES

1.Indrieri, A., et al., EMBO Mol Med, 2013. 5(2): p. 280-93.
2.Cacchiarelli D, et al., Cell. 2015 Jul 16;162(2):412-424.

## ACKNOWLEDGEMENTS

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