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The transcription factor FOSL1 exerts an indisputable role in KRAS-driven cholangiocarcinoma

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Brief introduction

Cholangiocarcinoma (CCA) is the second most commonly diagnosed primary liver tumour. Late diagnosis and the refractory nature of CCAs to conventional chemotherapies compromise the effectiveness of therapeutic options. Thus, CCA is ahigh mortality cancer that still lacking effective therapies for its treatment.

At the genomic level, *KRAS* is one of the most frequently mutated oncogene in CCA. More importantly, patients harboring mutations in *KRAS* present with the worst survival outcome. Our group previously developed a multimodal strategy integrating cross-species transcriptome analyses and a bioinformatics approach of clinical data across various tumor types (which spanned cholangiocarcinoma, lung cancer, pancreatic cancer, colon cancer and multiple myeloma)to unveil cancer vulnerabilities dependent on *KRAS* oncogene. A common transcriptional gene signature was identified, which included genes with a clinical and functional role in mutant *KRAS* lung and pancreatic adenocarcinoma and cholangiocarcinoma, such as the transcription factor *FOSL1* (Vallejo et al., Nature Communications, 2017).

Our hypothesis is that members of this multi-tumor *KRAS* signature could also have a clinical and functional role in other mutant *KRAS*-driven tumors such as CCA and, therefore, understanding the cellular and molecular mechanisms regulated by these genes could yield new therapeutic approaches for the treatment of this devastating disease

Objectives

1) To characterize the expression of a multi-tumor *KRAS* gene signature in CCA and its relationship with KRAS mutational status and patient survival.

2) To determine the functional role of a member of this signature, *FOSL1*, in human and mouse through experimental models *in vitro* and *in vivo*.

2)To define the impact of *FOSL1*conditional depletion in CCA carcinogenesis using an experimental genetically-engineered mouse models(GEMM) based on *KRAS* mutation and *Trp53* loss.

3) To unveil the molecular mechanisms triggered by *FOSL1* in *KRAS* mutant CCAs via transcriptome profiling and loss-of-function experiments of putative transcriptional targets.

Methodology

A CCA patient cohort (Andersen et al),with information on *KRAS* mutational status, gene expression and clinical information, was queried to investigate expression of a multi-tumor KRAS gene signature.

Human mutant and wild-type *KRAS* CCA cell lines, immortalized human and mouse cholangiocytes, and mouse mutant *KRAS* cell lines were used to investigate expression of the multi-tumor *KRAS* gene signature. Gain- and loss-of-function experiments to modulate *FOSL1*

expression were deployed using genetic approaches based on over-expression of tagged-constructs and RNAi/CRISPR-Cas9 technologies. ShRNAs and sgRNAs were designed and tested *in vitro* for maximal knockout efficiency. *FOSL1* expression was assessed by qPCR, Western blot and immunohistochemistry.

Experimental approaches *in vitro* included cell proliferation and colony-forming assays, and organoid cultures. Flow cytometry was used to study apoptosis and cell cycle alterations upon *FOSL1* inhibition. Experimental approaches *in vivo* were based on xenograft models derived from FOSL1-depleted human cell lines.

A previously described GEMM of cholangiocarcinoma, based upon *Kras* mutation and *Trp53* loss was deployed to conditionally induce*FOSL1* depletion using a liver specific Cre strain (*Kras*$^{G12D/wt}$;*Trp53*;Alb-Cre) in order to investigate its effect on tumor burden and mice survival. Furthermore, biochemical parameters of liver damage were determined in both control (*Kras*$^{G12D/wt}$;*Trp53*$^{f/f}$;Alb-Cre) and *FOSL1* knockout mice (*Kras*$^{G12D/wt}$;*Trp53*$^{f/f}$;*Fosl1*$^{f/f}$;Alb-Cre).

RNA-sequencing analysis was performed using a mutant *KRAS* CCA cell line where FOSL1 was genetically inhibited to establish a FOSL1 dependent gene regulatory network with putative targets harboring promoter binding sites for this AP1 transcription factor.

Results

Analysis of clinical data revealed that the multi-tumor KRAS gene signature predicts *KRAS* status and is a marker of poor survival in human CCA patients. A member of this gene signature, FOSL1, was shown to be upregulated in CCA patients harbouring KRAS mutations with regard to non-mutant ones and healthy donors. Similarly, FOSL1 was preferentially over-expressed in human CCA cell lines where KRAS was mutated. Furthermore, Fosl1 was found upregulated with regard to normal cholangiocites in a CCA GEMM driven by concomitant activation of mutant Kras and loss of Trp53. The differential FOSL1 expression based on the existence of KRAS mutations was demonstrated in human CCA and human cholangiocytes, where KRAS knockdown led to FOSL1 down-regulation and exogenous mutant KRAS transduction enhanced FOSL1 expression. Likewise, in tune with these human data, endogenous activation of mutant Kras in primary mouse cholangiocytes also induced Fosl1.

At the functional level, FOSL1 knockdown/knockout in human and mouse CCA cells induced a dramatic decrease in cell growth and clonogenic efficiency, and a prominent increase in cell apoptosis *in vitro*. Furthermore, FOSL1 depletion led to a marked reduction in the average volume of xenografted tumors derived from human and mouse CCA cells. Notably, FOSL1 inhibition in established tumors unveiled a dramatic delay in tumor growth, suggesting a potential role as a molecular target. The anti-tumor effect of FOSL1 depletion was further demonstrated in a GEMM of CCA, where FOSL1 conditional knockout extended mice survival with regard to Fosl1-expressing mice.

Mechanistically, a FOSL1-dependent gene-expression signature has been generated and queried for its clinical implication in CCA. Moreover, Chromatin Immunoprecipitation analyses are underway to identify FOSL1 downstream targets that may have a functional role in CCA.

Conclusions

Our work shows that FOSL1 expression is tightly regulated by KRAS oncogene in different stages of CCA tumourigenesis and, also, that FOSL1is necessary for normal homeostasis of CCA i*n vitro* and *in vivo*, through regulation of a transcriptional network whose functional implications are currently being studied. Therefore, FOSL1 represents an oncogene vulnerability on CCA driven by KRAS mutations that may unveil novel interventional strategies for this deadly cancer.