HEREDITARY ENDOCRINE CANCER GROUP

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OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features we have been able to propose diagnostic and prognostic markers, to identify altered pathways that could be therapeutically targeted, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying targeted and whole-exome next-generation sequencing to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

"We identified a new susceptibility gene for paraganglioma, discovered predictive markers of mTORi response, and uncovered the Hsa-miR-139-5p/HNRNPF axis as a critical modulator of thyroid tumour virulence."

RESEARCH HIGHLIGHTS

Recurrent germline *DLST* mutations in patients with multiple pheochromocytomas and paragangliomas (PPGLs). Taking as a starting point the involvement of the TCA cycle in PPGL development, we aimed to identify novel disease-related genes involved in this key metabolic pathway that could explain additional patients lacking mutations in known susceptibility genes. To this end, targeted sequencing of thirty-seven TCA cycle-related genes was applied to DNA from 104 PPGL patients with no mutations in the major known predisposing genes. In order to decipher the role of the identified variants, omic-based analyses, TCA-related metabolite determination and $^{13}C_5$-glutamate labelling assays were performed. We identified *DLST* germline variants in ~7% of patients. A recurrent mutation, p.Gly374Glu, found in 43% of patients, triggered accumulation of 2-hydroxyglutarate, both in tumours and in a heterologous cell-based assay designed to functionally evaluate *DLST* variants. p.Gly374Glu-DLST-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive DLST immunostaining not only in *DLST*-mutated tumours, but also in other tumours in which the TCA cycle was disrupted. In summary, this study reveals *DLST* as a new PPGL susceptibility gene and further strengthens the relevance of the TCA cycle in PPGL development.

Mutations leading to extraordinary responses to mTOR inhibitors. The inhibitors of the mammalian target of rapamycin (mTOR) are key drugs for the treatment of several tumours. However, we lack markers able to identify patients with enhanced treatment sensitivity. To discover molecular determinants of drug response and to contribute to the definition of predictive biomarkers, we recruited renal cancer patients with extraordinary responses to these drugs and performed a comprehensive genomic, immunochemical and functional characterisation of the tumours. In two young adults with metastatic cancer, a renal epithelioid angiomyolipoma (EAML) and a chromophobe renal cell carcinoma, that upon rapalog treatment had a complete response at metastatic sites and durable responses, we could identify *TSC2* somatic mutations as causative of the extraordinary responses. These findings support a high efficacy of mTOR inhibitors in malignant EAML and in a subset of patients with chromophobe renal cancer, and propose sequencing of mTOR pathway genes to guide therapy with these drugs.

Deep sequencing of small RNAs reveals a prognosis marker functionally associated with alternative splicing modulation in thyroid cancer. It is urgent to identify biomarkers and functional networks associated with aggressive thyroid cancer in order to anticipate disease progression and facilitate patient-personalised management. The miRnome sequencing of thyroid tumour series enriched for advanced disease patients uncovered miRnome profiles correlated with tumour-specific histopathological and molecular features, such as stromal-cell infiltration and tumour-driver mutation. Differential analysis considering disease prognosis revealed a consistent hsa-miR139-5p down-expression in primary carcinomas from patients with recurrent/metastatic disease. Exogenous expression of hsa-miR-139-5p significantly reduced migration and proliferation abilities of anaplastic thyroid cancer cells. Proteomics analysis pointed to *RICTOR*, *SMAD2/3* and *HNRNPF* as hsa-miR-139-5p putative targets *in vitro*.

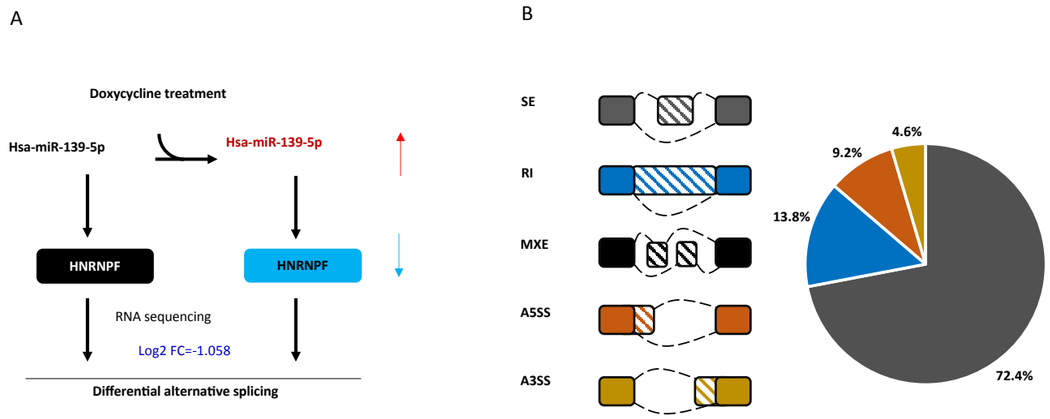

Figure Hsa-miR-139-5p/HNRNPF axis modulates gene-transcripts balance. (**A**) Alternative splicing analysis experiment. DeSeq2 differential expression analysis showed a reduction of *HNRNPF* mRNA abundance (Log2 FC=-1.058) upon hsa-miR139-5p expression induction. rMATS method identified differences in alternative splicing. (**B**) Events with a significant different inclusion level (FDR<0.05) upon hsa-miR-139-5p/HNRNPF axis regulation. Analysis considers junction and target exon counts from RNA sequencing data. SE: Skipped exon, MXE: Mutually exclusive, A5SS: Alternative 5' splice site, A3SS: Alternative 3' splice site; RI: Retained intron.

Significantly, mRNA abundance of *HNRNPF*, an alternative splicing factor mainly involved in cryptic exon inclusion/exclusion, showed an anti-correlation with hsa-miR-139-5p expression in human tumours. Analysis of alternative splicing from RNA sequencing data revealed 174 events differentially regulated upon *HNRNPF* repression in genes and signalling cascades critical for thyroid cancer (FIGURE). These results point at hsa-miR139-5p/HNRNPF/gene-transcripts balance as a novel regulatory axis associated with tumour virulence and modulation of major thyroid cancer signalling pathways. ■

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AWARDS AND RECOGNITION

- Mercedes Robledo, Group Leader of the 706 Unit, CIBERER (*Centro de Investigación Biomédica en Enfermedades Raras*). Research Programme on Hereditary Cancer, Haematological and Dermatological diseases.