Mesenteric fibrosis in small intestinal neuroendocrine tumors

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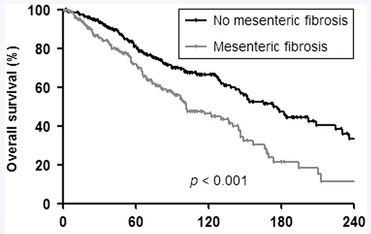
INTRODUCTION

Mesenteric fibrosis (MF) in patients with small intestinal neuroendocrine tumors (SI-NETs) causes significant morbidity and mortality and is poorly understood. MF occurs in up to 50% of SI-NET patients and is caused by a metastatic lesion circumscribed by an extensive fibrotic reaction in the mesentery. There are no biomarkers or radiological criteria to predict complications of SI-NET associated MF. Due to this lack of knowledge, the UCL/Royal Free London and Erasmus MC Rotterdam, are collaborating to utilise skill sets specific to each centre to then integrate and validate the understanding of MF pathogenesis.



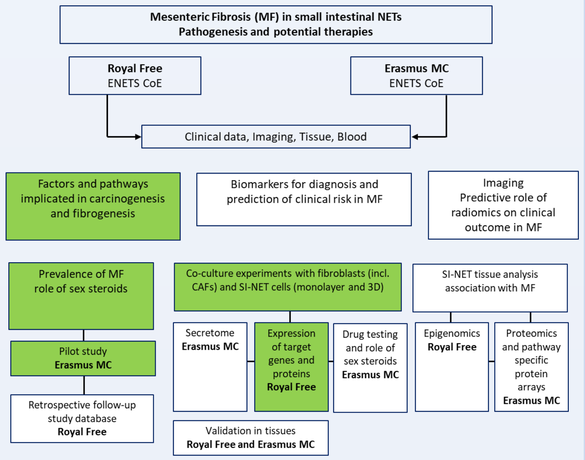
Difference in overall survival of SI-NET patients with and without mesenteric fibrosis from Blazevic *et al.* Mesenteric fibrosis and palliative surgery in small intestinal neuroendocrine tumours, Endocrine-Related Cancer, 2018[1]

OBJECTIVES

The aim of this study is to identify target genes that will help to determine the pathogenesis of MF in SI-NETs, as well as understanding the crosstalk between tumour cells and cancer-associated fibroblasts during the development of MF.

See below:

OUTLINE OF COLLABORATIVE RESEARCH PROGRAMME INTO MESENTERIC FIBROSIS



RESULTS: Prevalence of mesenteric metastasis and associated fibrosis, role of sex steroids

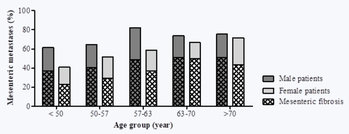


Figure 1. Sexual dimorphism in prevalence of mesenteric metastasis in SI-NETs. The patient cohort (N = 559) was divided into five equal age groups: < 50 years, 50 – 57 years, 57 – 63 years, 63 – 70 years, > 70 years.

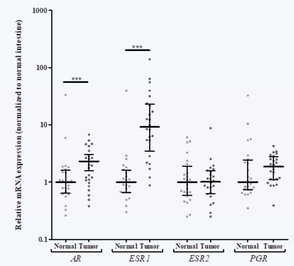


Figure 2. *AR, ESR1, ESR2* and *PGR* mRNA expression in 24 primary SI-NETs (tumor) and adjacent normal intestinal tissue (normal). Scatter plot with individual data points shown with median (wide line) and interquartile range (narrow lines). *** $P < 0.001$ Primary SI-NET vs normal intestinal tissue.

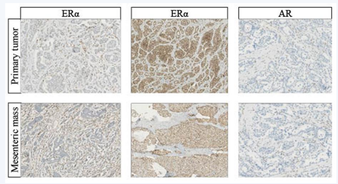


Figure 3. Immunohistochemical analysis of ERα and AR expression in primary tumors and paired mesenteric masses.

Mesenteric metastases and fibrosis are more prevalent in men (71%) than women (58%, p=0.001). In women, prevalence of mesenteric metastases increased gradually with age from 41.1% in women <50 years to 71.7% in women >70 years.
Increased expression of ESR1 and AR mRNA in primary tumors compared to healthy intestine.

RESULTS: Models to study interaction between NET cells and fibroblasts

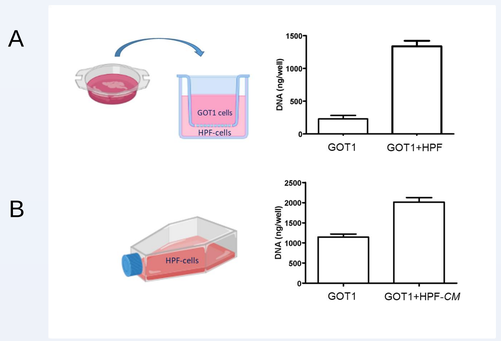


Figure 4. Interaction between NET cells and fibroblasts will be studied using different approaches. A: in a transwell co-culture model of fibroblasts and NET cells (example HPF-5 cells stimulate growth of GOT-1 cells, B: using conditioned medium of fibroblasts (HPF-*CM*) and NET cells (example conditioned medium of HPF-5 cells stimulates growth of GOT-1 cells), C: using a spheroid model with cell-cell contact of fibroblasts and NET cells (example GOT-1 spheroid at different stages of culture). Similar studies will be performed for primary NETs and CAFs.

RESULTS: transcriptomic analysis of GOT1 and LX2 cell interaction in a paracrine cell culture model

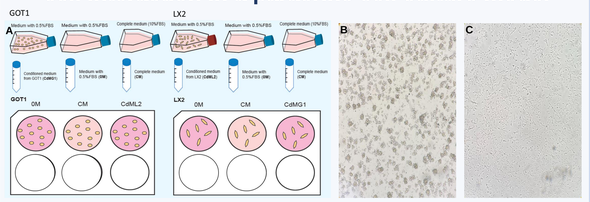


FIGURE 5. (**A).** Diagram showing the different culture conditions of the cell lines. Cells were treated grown in medium with 0.5%FBS (0M), 10%FBS (CM) and conditioned medium (CdM), followed by RNA extraction and sequencing. Analysis was performed by Gene set enrichment analysis (GSEA). **(B)** GOT1, a midgut NET cell line and **(C)** Hepatic Stellate Cell line LX2.

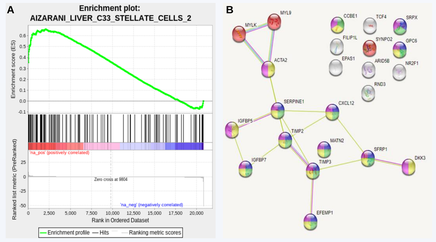


Figure 6. **(A)** Enrichment plot of Reactome: Aizarani Liver C33 stellate cells from a GSEA analysis. Condition: CdMG1toL2 vs 0MtoL2 .**(B)** a diagram of protein interactions generated using STRING analysis software.

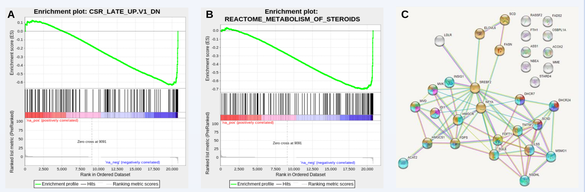


Figure 7. (**A**) Enrichment plot of Reactome: CSR late up, (**B**) and Reactome: Metabolism of steroids from a GSEA analysis. Condition CdML2toG1 vs 0MtoG1. (**C**) Diagram of protein interactions generated using STRING analysis software.

Figure 8. **(A)** Enrichment plot of Reactome: Fatty acid metabolism and **(B)** Reactome: Cholesterol biosynthesis from a GSEA analysis. Condition: CMtoL2 vs CdMG1toL2. (**C**) Diagram of protein interactions generated using STRING analysis software.

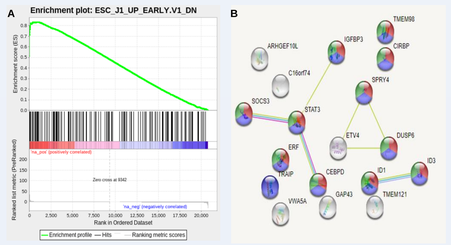
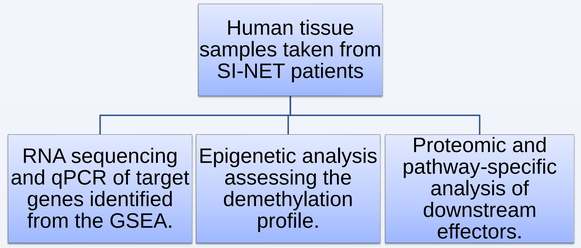


Figure 9. **(A)** Enrichment plot of Reactome: ESC from a GSEA analysis. Condition: CMtoG1 vs 0MtoG1. **(B)** Diagram of protein interactions generated using STRING analysis software. .

CONCLUSION

- Sexual dimorphism in SI-NET patients was most pronounced in mesenteric disease and the risk of mesenteric metastasis in women increases around menopause. The combination of increased ERα and AR expression in the SI-NET microenvironment suggests a modulating role of sex steroids in the development of the mesenteric metastasis and fibrosis of SI-NETs.
- Unravelling the interactions between SI-NET cells and CAFs and assessment of the effects of different stimuli on fibrogenesis will provide insight into the pathogenesis of MF. This could result in the development of biomarkers for MF and identify therapeutic targets to inhibit cell growth and fibrosis.
- Combining drugs with different modes of action may inhibit release of serotonin and other mediators involved in fibrogenesis more effectively resulting in a significant improvement for patients with SI-NET with MF.

ONGOING EXPERIMENTS



- Study the role of sex steroids on NET-fibroblast interaction.
- Secretome analysis of NET-fibroblast interaction.
- The predictive role of radiomics on clinical outcome on MF in patients who are asymptomatic vs. those who need acute surgery during follow-up in a large patient group.

REFERENCES

1. Blazevic et al. Mesenteric fibrosis and palliative surgery in small intestinal neuroendocrine tumours. *Endocr. Relat. Cancer., 2018.*

2. Blazevic et al. Evolution of the Mesenteric Mass in Small Intestinal Neuroendocrine Tumours. *Cancers 2021.*

3. Blazevic et al. Sexual dimorphism in small-intestinal neuroendocrine tumors: lower prevalence of mesenteric disease in premenopausal women. *Submitted.*

ACKNOWLEDGEMENTS & CONTACT

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