Metformin Inhibits Small Intestinal Neuroendocrine Tumor (SI-NET) Proliferation In Vivo

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Introduction

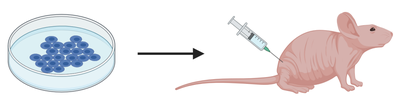
- Small intestinal neuroendocrine tumors (SI-NETs) are rare and slow-growing, with most patients being diagnosed at a late stage with distant metastases.
- Metformin has been hypothesized as a potential anti-tumor agent by several studies in the past few years.
- Confirmed by experimental studies in which metformin has inhibited cancer cell growth in different cancers.
- We recently demonstrated that metformin treatment repressed the cell viability of SI-NET cells and inhibited the proliferation of cell spheroids.

Aim

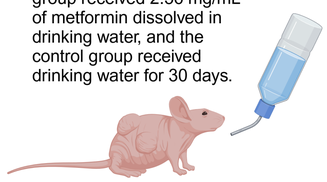
- To investigate the effect of metformin on the proliferation of SI-NETs in vivo, to identify novel molecular targets, and to unravel mechanisms responsible for the anti-tumor activity of metformin.

Materials and methods

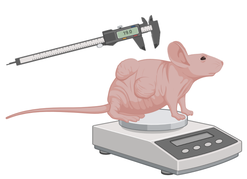
1. Cells were resuspended and subcutaneously injected in the hind flank of the NMRI-nude mice, which produced palpable xenograft tumors.



2. The metformin treatment group received 2.56 mg/mL of metformin dissolved in drinking water, and the control group received drinking water for 30 days.

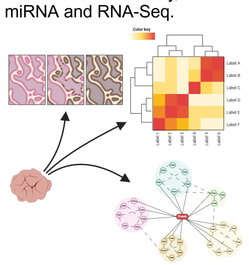


3. Tumor growth was monitored weekly by caliper measurement and mouse body weight.



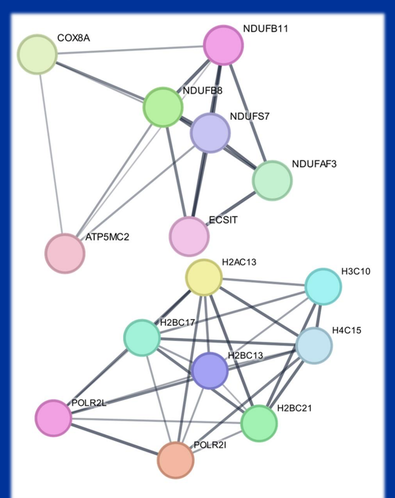
4. After 30 days of treatment, the mice were euthanized, and the xenograft tumors were dissected.

5. Tumor sections were subjected to immunohistochemistry, miRNA and RNA-Seq.



Conclusion

- **Metformin inhibited proliferation** and **Ki-67** expression in **SI-NET xenografted tumors.**
- ***CST3*** (Cystatin C) expression was found to be **induced** by **metformin**.
- ***CST3*** is **inhibited** in **SI-NET** compared to **EC** cells.
- ***CST3*** is a potential **clinically relevant** target for further functional studies.
- Overlapping gene clusters:
  - **Oxidoreductase**
  - **Nucleosome-RNA polymerase II**



Results

- A **smaller tumor size** could be observed in the metformin treatment groups (Fig. A).
- All the tumors (n = 22) stained positively for the neuroendocrine cell marker **Synaptophysin**, and the expression of **Ki-67** was reduced in the tumor tissues while no effect on **activation of caspase-3** was detected (Fig. B).
- **Significantly** ($p \leq 0.05$) inhibited ***CD44*** expression were identified in both **CNDT2.5** and **GOT1** xenografted tumors, indicating successful metformin treatment.
- **227** significantly ($p \leq 0.05$) differentially expressed genes (DEGs) overlapping between **CNDT2.5** and **GOT1** xenografted tumors could be detected, and one significantly ($p \leq 0.05$) differentially expressed miRNA: **hsa-miR-99a-5p**.
- We could observe two overlapping gene networks: an **oxidoreductase** and a **nucleosome-RNA polymerase II** cluster.
- We compared our **227** genes against **overlapping** significantly differentially expressed genes in our in-house (unpublished) dataset consisting of comparisons between patient-derived enterochromaffin (**EC**) cells and **SI-NET** cells by single-cell RNA-seq (**scRNA-seq**).
- This revealed ***CST3*** (Cystatin C) as a potential clinically relevant target for further functional genomic studies. This is due to its expression being significantly **inhibited** in **SI-NET**, and our results revealed that ***CST3*** expression levels were significantly **induced** in both our **CNDT2.5** and **GOT1** xenografted tumors following **metformin treatment** (Fig. C).