Targeting MUC1-C in pancreatic neuroendocrine tumor

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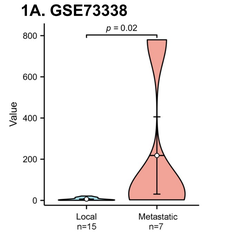
Background

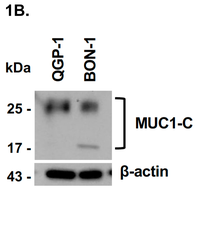
The MUC1 gene evolved in mammals for adaptation of barrier tissues to loss of homeostasis. Dependence on the oncogenic MUC1-C subunit for the cancer stem cell (CSC) state, self-renewal capacity and tumorigenicity has been uncovered across pan-cancers, including neuroendocrine prostate cancer (NEPC) (Yasumizu Y, 2020), small cell lung cancer (SCLC) (Fushimi A, 2022) and Merkel Cell Cancer (MCC) (Morimoto Y, 2022). However, there is no known involvement of MUC1-C in the pathogenesis of pancreatic neuroendocrine tumors (pNETs).

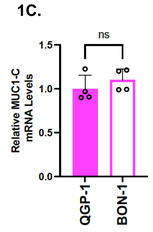
Results

pNET cells are dependent on MUC1-C for clonogenic survival.

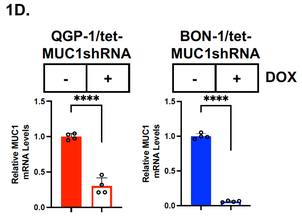
- Analysis of a pNET dataset (GSE73338) demonstrated that the *MUC1* gene is significantly upregulated in metastatic as compared to localized tumors, indicating that the MUC1-C subunit may contribute to pNET progression (Fig. 1A).
- The widely studied QGP-1 and BON-1 pNET cell lines express MUC1-C transcripts and protein (Fig. 1B; 1C).

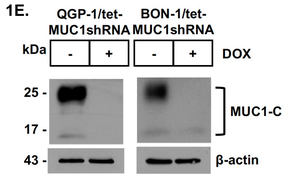


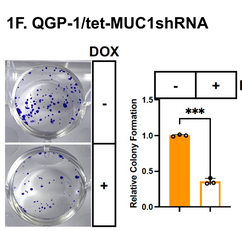


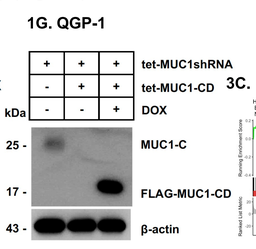


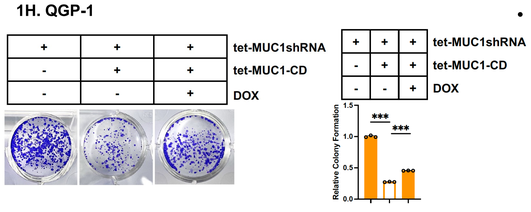
- We established QGP-1 and BON-1 cells transfected with a control tet-CshRNA or a tet-MUC1shRNA. DOX treatment of QGP-1/tet-MUC1shRNA and BON-1/tet-MUC1shRNA cells downregulated MUC1-C mRNA (Fig. 1D) and protein (Fig. 1E) levels.
- Silencing MUC1-C in QGP-1 cells suppresses their capacity for forming colonies (Fig. 1F)
- As confirmation of MUC1-C dependence, we rescued MUC1-C silencing with inducible expression of the MUC1-C cytoplasmic domain (MUC1-CD) (Fig. 1G), which reversed the loss of clonogenicity (Fig. 1H).





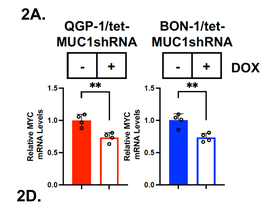


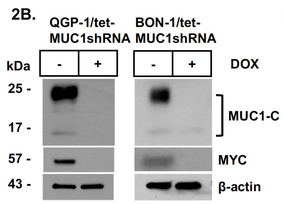


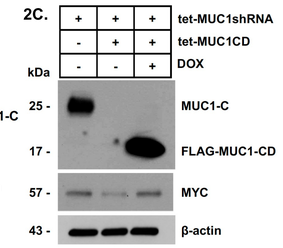


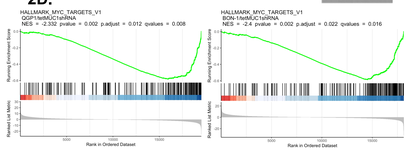
MUC1-C regulates MYC in pNET cells.

- Dysregulation of MYC is a common feature in pNET tumors (Yang K, 2021).
- Silencing MUC1-C in QGP-1 and BON-1 cells decreased MYC mRNA (Fig. 2A) and protein (Fig. 2B) levels. Additionally, rescue of MUC1-C silencing with MUC1-CD reversed the downregulation of MYC expression (Fig. 2C).
- In RNA-seq, GSEA of the QGP-1 and BON-1 genesets uncovered involvement of MUC1-C in regulating the HALLMARK MYC TARGETS V1 signature (Fig. 2D).



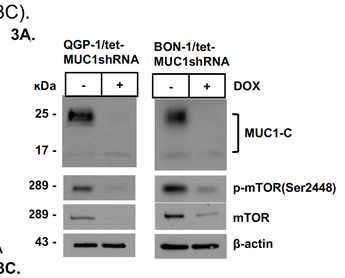


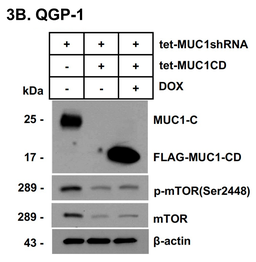


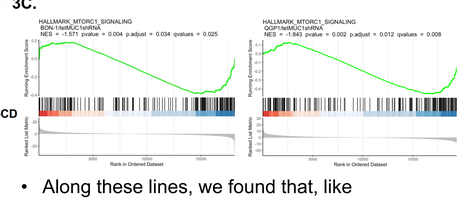


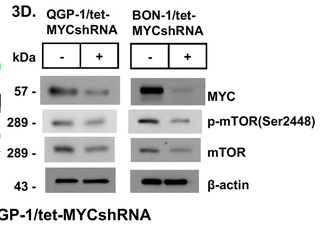
MUC1-C/MYC signaling regulates the mTOR1 pathway

- Dysregulation of mTORC1 is widely recognized in pNET tumors and is a target for treatment with everolimus (Yang K, 2021).
- MUC1-C silencing in QGP-1 and BON-1 cells results in downregulation p-mTOR(SER2448) and mTOR levels (Fig. 3A), which were rescued in part by MUC1-CD expression (Fig. 3B).
- GSEA further demonstrated that silencing MUC1-C in QGP-1 and BON-1 cells significantly associates with suppression of the HALLMARK MTORC1 SIGNALING gene signature (Fig. 3C).

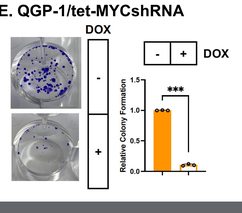






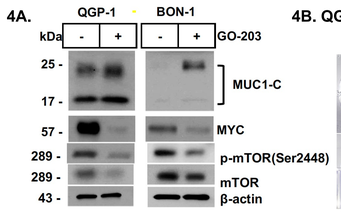


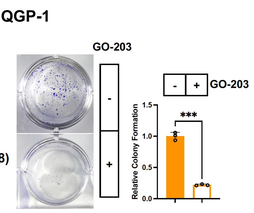
- Along these lines, we found that, like MUC1-C, silencing MYC decreases p-mTOR(SER2448) and mTOR levels (Fig. 3D). In addition, like MUC1-C, silencing MYC suppressed QGP-1 colony formation (Fig. 3E), indicating that MUC1-C/MYC signaling regulates effectors of the mTORC1 metabolic pathway in association with driving clonogenic survival.

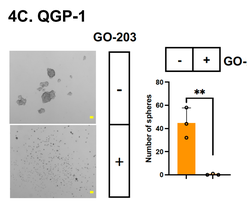


Targeting MUC1-C with the GO-203 inhibitor suppresses MUC1-C/MYC signaling, self-renewal and tumorigenicity.

- Treatment with the GO-203 inhibitor, which blocks MUC1-C function, similarly suppressed (i) mTOR and MYC expression (Fig. 4A), and (ii) colony and tumorsphere formation (Fig. 4B; 4C).

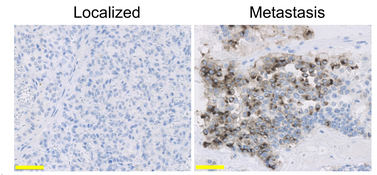




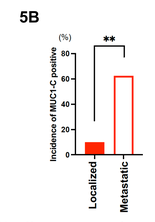


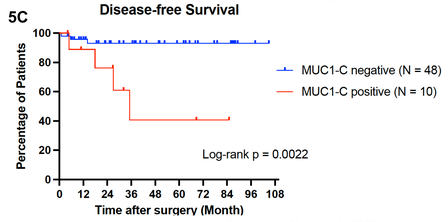
Association of MUC1-C expression in pNET tumors with adverse clinical outcomes.

- We analyzed expression of MUC1-C by IHC in surgically resected pNETs from 58 patients.
- Upregulation of MUC1-C staining in primary tissue samples from pNET patients with metastatic vs localized by IHC (Fig. 5A).



- The positive rate of MUC1-C was significantly higher in the primary tumor with metastasis than in that with localized tumor (63% vs 10%, p = 0.002) (Fig. 5B).
- Survival analyses by the Kaplan–Meier method showed that patients with MUC1-C in their tumors have a shorter disease-free survival (p = 0.0022) (Fig. 5C).





- Studies are underway that asses the effect of targeting MUC1-C in pNET xenograft models.

Conclusion

Our results demonstrate that (i) pNET cell lines are addicted to MUC1-C, and (ii) MUC1-C expression is increased in metastatic pNET tissues. These findings indicate that MUC1-C represents a potential target for advancing the treatment of patients with metastatic pNETs with the anti-MUC1-C agents that are under clinical and preclinical development.

References

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