

# B-8

## Deciphering the Functionality of MAX Phosphorylation Dynamics in Pancreatic Neuroendocrine Tumors

*PTheo Hines, Mourad Bendjennat, Rachael Guenter, Herbert Chen, J. Bart Rose, Andrea Gillis.*

*Department of Surgery, University of Alabama at Birmingham.*

### BACKGROUND

Pancreatic neuroendocrine tumors (PNETs) are the second most common pancreatic malignancy, with patients exposed to adverse social determinants of health (aSDOH) exhibiting worse outcomes (larger tumors and higher rates of lymph node metastasis). We identified Myc associated factor X (MAX) expression to be altered in adversity. MAX regulates the activity of the Myc oncogene, and its dysregulation enhances aggressive growth of some cancers, but its effect remains unstudied in PNETs. The transcriptional activities of MAX can be altered by phosphorylation at residue Serine 11, but the function of Serine 2 phosphorylation remains elusive. This study aims to design tools to evaluate the biological role of the phosphorylation dynamics of MAX in PNETs.

### METHODS

MAX and phospho-MAX Ser11 were evaluated using western blotting in HPNE, a normal immortalized human pancreatic cell line, versus human PNET cell lines: BON-1 and QGP-1. Additionally, recombinant vectors expressing eGFP-tagged phospho-mutants of MAX individually and in combination (S2A, S2D, S11A, S11D, S2A+S11A, S2A+S11D, S2D+S11A, S2D+S11D) were constructed using site-directed mutagenesis. These mutants were expressed in BON-1 cells and evaluated for correct size and cellular localization using western blotting and fluorescence microscopy, respectively. Finally, BON-1 cells transfected with our eGFP-MAX variants were selected using geneticin for ~3 weeks, then imaged using fluorescence microscopy to confirm colony formation.

### RESULTS

On western blotting we confirmed that MAX Ser11 is phosphorylated in HPNE cells, but not in BON-1 and QGP-1. Compared to wild type MAX, all N- and C- terminally tagged phospho-mutants showed identical reactivity to anti-GFP and anti-MAX antibodies and similar nuclear cellular localization according to fluorescence microscopy. Furthermore, after geneticin selection, we confirmed that BON-1 cells proliferated similarly while overexpressing our GFP-tagged MAX phospho-mutants, ruling out cytotoxicity.

### CONCLUSIONS

We successfully generated the necessary tools to explore our novel adversity associated gene: MAX. Future ex vivo experiments will evaluate growth curve rates of MAX phospho-mutants and the effects of MAX phosphorylation on its transcriptional network (Myc and Mad). Our study of MAX and its phosphorylation dynamics could validate a new biomarker for prognosis and a novel therapeutic target for treatment of patients with PNETs, reducing health disparities.

**ABSTRACT ID 33449**

NANETS 2025 Symposium Abstracts