### Introduction

The epidermal growth factor receptor (EGFR) gene is amplified/mutated in >50% of glioblastomas (GBMs). Its product receptor protein is a driver of GBM growth. Unfortunately, EGFR-TKIs have failed to inhibit GBM growth due to existing convergent signaling pathways. The substance P (SP) receptor (NK1R) has been shown to mimic EGFR function in GBM cells. Using proteomic-profiling techniques, we examined the degree of convergence between these pathways.

### Methods

U373 GBM cells, known to express both receptors, were maintained in MEM media supplemented with non-essential amino acids, sodium pyruvate, and 10% fetal bovine serum (FBS). Cells were dissociated with 0.25% trypsin and plated in a six-well plate at a density of 500,000 cells/well in serum-free media (replaced at 24 hrs) and incubated for an additional 24 hrs at 37°C and 5% CO₂ prior to treatment with either PBS, 100 ng/mL EGF, or 100 nM SP. After 10 min, cells were washed twice with ice-cold PBS, lysed following protocols reported by the Reverse Phase Protein Arrays (RPPA) core facility at M.D. Anderson, lysate protein concentrations adjusted to 1.5 mg/mL, and samples stored at -800°C prior to RPPA analysis.

### Results

RPPA analysis demonstrated the expression of 15 of 297 proteins/phosphoproteins to be increased in U373 GBM cells after EGF administration compared to controls. Interestingly, the increase in expression of 9 of these 15 proteins (60%) was again seen after SP administration. (ACC_pS79, Akt_pS473, Akt_pT308, HSP27_pS82, MAPK_pT202_Y204, mTOR, p38_pT180_Y182, p44-42-MAPK, p90RSK_pT573). SP did not affect the expression of the remaining five proteins (EGFR_pY1068, Histone-H3, NDRG1_pT346, PTEN, and TAZ). Finally, the expression of fibronectin was increased with EGF but decreased with SP.

### Conclusion

EGFR and NK1R share ~60% of their signaling transduction pathways in U373 GBM cells. Therefore, we hypothesize that combination therapy to block both receptors may yield more promising results over single agents.