NF1-mutated tumors exhibit increased sensitivity to autophagy inhibition

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BACKGROUND

• Loss of functional NF1 gene leads to constitutive activation of the Ras/MAPK pathway which induces cellular proliferation and tumorigenesis.
• The NF1 phenotype involves large plexiform neurofibromas that usually cannot be treated surgically as the lesions are likely to be extensively involved with the affected nerve and have a propensity to recur after resection.
• Despite the recent expansion of kinase inhibitors (including MEK inhibition) for treatment of NF1 mutated tumors there remains a risk of chemo-resistance in kinase targeted therapies.
• Autophagy, a heavily regulated process by which cellular waste is transferred to lysosomes for degradation and processing, is an integral part of tumor cell survival under stressful conditions including nutrient deprivation and chemotherapy. Autophagy has been demonstrated to play an important role in chemo-evasion in other tumor types with MAPK pathway dysregulation but has yet to be explored in NF1-mutated tumors.

METHODS

• A CRISPR/Cas9 mediated NF1 KO was derived from human immortalized Schwann cells.
• Autophagy inhibition was achieved pharmacologically by chloroquine (CQ) and genetically via shRNAi of ATG5.
• Trametinib was used for MEK inhibition.
• In vitro viability assays:
  - CellTiter-Glo® Luminescent Cell Viability Assay
  - Long-term survival colony formation assay
  - Incucyte growth measurement assay was used to assess cellular growth
• Western blot analysis was used for protein expression analysis

RESULTS

A.

B.

C.

CONCLUSIONS

• HSC cells with NF1-KO exhibit upregulation of the MAPK pathway
• Non-BRAF dysregulation within the MAPK pathway yields increased autophagic activity
• Under serum starvation stress, NF1 KO cells upregulate autophagy to a greater extent than NF1 WT cells.
• Inhibition of autophagy via genetic inhibition or chloroquine increases sensitivity to MEK inhibition

IMPLICATIONS

Autophagy inhibition via CQ may be an effective adjunctive treatment for NF1 mutated tumors and suggests that diverse CNS tumor types with MAPK pathway dysregulation are susceptible to autophagy inhibition

DISCLOSURES

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