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Inhibition of ULK1/2-Mediated Autophagy Augments Antigen Processing and Presentation in STK11-mutant NSCLC

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Therapeutic Hypothesis: ULK Inhibition Will Decrease Autophagy and Restore Antigen Processing and Presentation in STK11mut NSCLC Tumors

- STK11 inactivating mutations and deletions are present in 9-20% of NSCLC [1]
  - STK11 is a tumor suppressor that encodes LKB1
  - Mutation or deletion of STK11 results in a lack of cellular LKB1 protein
- STK11mut/del results in immunologically cold tumors that respond poorly to therapy, with a 5-10% objective response rate to PD-1 blockade, despite having high tumor mutational burden (TMB) and neoantigen load [2-4]
- STK11mut/del in NSCLC is associated with higher levels of ULK-mediated macroautophagy [3]
- Macroautophagy is a cellular degradation and recycling progress that removes cytoplasmic contents and organelles through lysosomal degradation [5]
  - ULK1/2 are serine/threonine kinases that complex with ATG13, FIP200, and ATG101 to initiate autophagy
  - The ULK complex phosphorylates ATG14 and activates the VPS34 complex
  - p62 traffics ubiquitinated cargo to phagophores and gets degraded in the autophagosome
  - LC3-II is a marker of autophagosomes
- High levels of autophagy in STK11mut/del NSCLC results in localization of MHC-I to autophagosomes and lysosomes leading to its degradation and decreased antigen presentation [6]

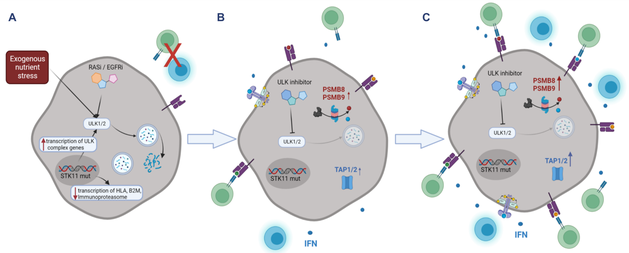
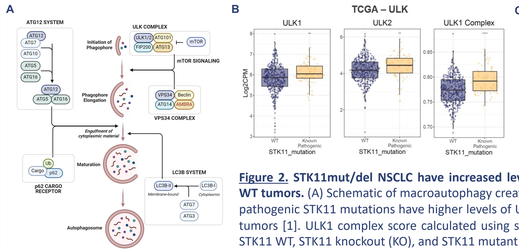


Figure 1. Therapeutic Hypothesis
A. ULK-dependent autophagy is upregulated in STK11-mutant tumors, which drives the downregulation of antigen presentation machinery. **STK11-mutant tumors have low neoantigen presentation despite high TMB.**
B. ULK1/2 inhibition will reverse autophagy-mediated downregulation of antigen processing and presentation machinery.
C. ULK1/2 inhibition in the presence of IFNγ will further improve immune "visibility" of the tumor and responses to therapy.

STK11mut/del NSCLC Have High Levels of ULK-Mediated Autophagy



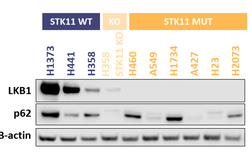
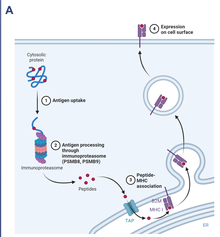
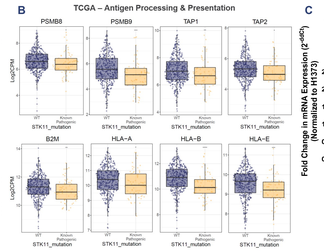
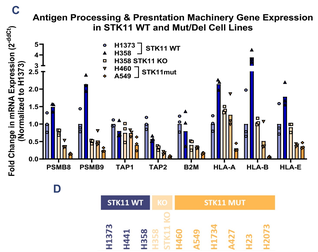


Figure 2. STK11mut/del NSCLC have increased levels of ULK-mediated autophagy compared to STK11 WT tumors. (A) Schematic of macroautophagy created with BioRender.com. (B) NSCLC tumors with known pathogenic STK11 mutations have higher levels of ULK1 complex genes compared to STK11 wildtype (WT) tumors [1]. ULK1 complex score calculated using ssGSEA method [7]. (C) LKB1 and p62 protein levels in STK11 WT, STK11 knockout (KO), and STK11 mutant (mut) NSCLC cell lines.

STK11mut/del NSCLC Have Low Levels of Antigen Processing and Presentation Machinery







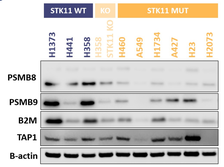


Figure 3. STK11mut/del NSCLC display decreased levels of antigen processing and presentation machinery (APM) compared to STK11 WT tumors. (A) APM schematic created with BioRender.com. (B) NSCLC tumors with known pathogenic STK11 mutations have lower levels of APM compared to STK11 WT tumors [1]. (C) Comparison of mRNA levels of APM genes in STK11 WT, STK11 KO, and STK11mut NSCLC cell lines (D) APM protein levels in STK11 WT, STK11 KO, and STK11mut NSCLC cell lines.

Double Knockout of ULK1 and ULK2 Decreases Autophagy and Increases Antigen Processing and Presentation in STK11mut NSCLC

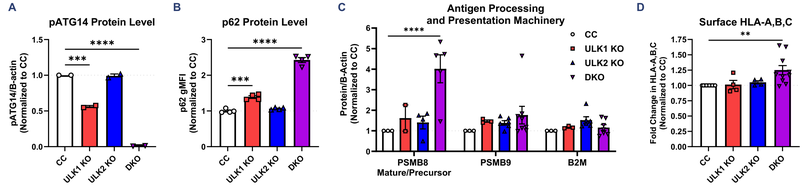


Figure 4. Double knockout of ULK1 and ULK2 decreases autophagy and increases APM in STK11mut NSCLC A549 cells. (A) ULK DKO reduces pATG14 to undetectable levels suggesting full inhibition of ULK-mediated autophagy. (B) ULK1 single KO and ULK DKO increase p62, but the increase with the DKO is significantly higher indicating stronger inhibition of autophagy. (C) ULK DKO increases PSMB8 mature/precursor ratio at the protein level. (D) ULK DKO increases cell surface HLA-A,B,C. ***Dual knockout of ULK1/2 is required to fully inhibit ULK-mediated autophagy and increase antigen processing and presentation machinery.***

ULK Inhibition Reduces Autophagy in STK11mut NSCLC

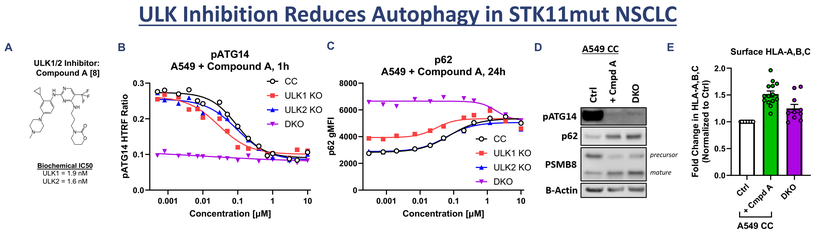


Figure 5. ULK1/2 inhibition reduces autophagy in STK11mut NSCLC A549 cells. (A) Tool ULK1/2 inhibitor Compound A synthesized following literature reported protocols [8]. (B) ULK inhibition with Compound A reduces pATG14 levels in A549 CC, ULK1 KO, and ULK2 KO to the same basal levels of ULK DKO. (C) ULK inhibition with Compound A increases p62 levels in A549 CC, ULK1 KO, and ULK2 KO to comparable basal levels of ULK DKO. (D) 48h of ULK inhibition with Compound A reduces pATG14 and increases p62 and PSMB8 mature/precursor ratio in A549 CC to the same basal levels as ULK DKO. (E) 48h of ULK inhibition with Compound A increases surface HLA-A,B,C levels to comparable basal levels of ULK DKO. ***ULK1/2 inhibition reduces autophagy and increases APM to comparable levels as CRISPR DKO of ULK1/2 in A549 cells.***

ULK Inhibition Restores Antigen Processing and Presentation in STK11mut NSCLC

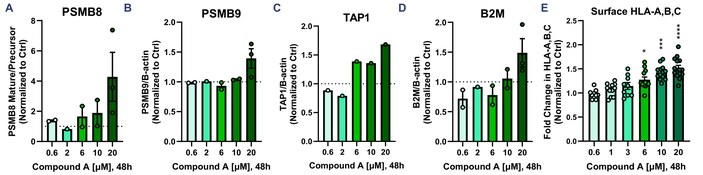
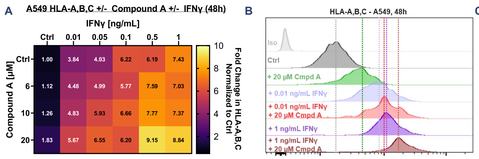
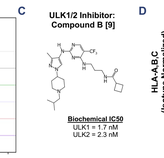


Figure 6. ULK inhibition increases APM in STK11mut NSCLC A549 cells. Fold change in (A) PSMB8 mature/precursor levels, (B) PSMB9 levels, (C) TAP1 levels, (D) B2M levels, and (E) cell surface HLA-A,B,C after 48h of ULK inhibition in STK11mut A549 cells. ***ULK1/2 inhibition reverses autophagy-mediated downregulation of antigen processing and presentation in STK11mut A549 cells.***

ULK Inhibition Combines with Low Levels of IFNγ to Further Increase Cell Surface MHC-I in STK11mut NSCLC





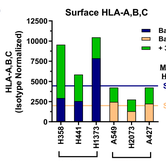
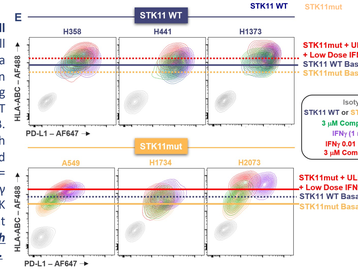


Figure 7. ULK inhibition combines with low levels of IFNγ to increase cell surface MHC-I levels in STK11mut NSCLC. (A) Fold change in A549 cell surface HLA-A,B,C after treatment with Compound A, low dose IFNγ, or a combination. (B) Representative HLA-A,B,C histograms as described in panel A. (C) Tool ULK inhibitor Compound B synthesized following literature reported protocols [9]. (D) HLA-A,B,C basal levels of STK11 WT and STK11mut cell lines and increases after treatment with Compound B. ULK inhibition increases STK11mut HLA-A,B,C to levels comparable with STK11 WT cell lines. (E) HLA-A,B,C and PD-L1 levels in STK11 WT and STK11mut cell lines after treatment with vehicle control (STK11 WT = navy, STK11mut = orange), 3 μM Compound B (green), 1 ng/mL IFNγ (purple), and low dose 0.01 ng/mL IFNγ + 3 μM Compound B (red). ULK inhibition combined with IFNγ increases HLA-A,B,C to a greater extent than PD-L1. ***ULK1/2 inhibition in STK11mut cell lines in combination with low dose IFNγ normalizes HLA-A,B,C levels to that of STK11 WT cell lines.***



Conclusions

- STK11mut/del NSCLC tumors have high levels of ULK-mediated autophagy that result in decreased antigen processing and presentation machinery (APM) at the transcriptional and protein levels.
- ULK1/2 double knockout in STK11mut A549 cells decreases autophagy and increases APM.
- ULK inhibition with a dual ULK1/2 inhibitor reduces autophagy and increases APM and surface HLA-A,B,C in STK11mut A549 cells.
- ULK1/2 inhibition combines with low dose IFNγ to restore APM in NSCLC STK11mut cell lines to levels similar to STK11 WT cells which are known to respond to immunotherapy.
- The data presented here provides a rationale for targeting ULK1/2 to amplify immune recognition in immunologically cold tumors with high autophagy to increase responses to immunotherapies.

References

[1] cBio Portal. [2] Ravi et al. (2023) Nature Genetics. Genomic and transcriptomic analysis of checkpoint blockade response in advanced NSCLC. [3] Deng et al. (2021) Nature Cancer. ULK1 inhibition overcomes compromised antigen presentation and restores antitumor immunity in LKB1-mutant lung cancer. [4] Skoulidis et al. (2018) Cancer Discovery. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. [5] Qian & Yang (2016) Oncotarget. Functional role of autophagy in gastric cancer. [6] Yamamoto et al. (2020) Nature. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. [7] Barbie et al. (2010) Nature. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. [8] Patent US11530206B2 to Deciphera Pharmaceuticals. [9] Patent US11159013AB2 to Deciphera Pharmaceuticals.