Integrative mass spectrometry and RNA-sequencing identifies DLK1 as a candidate immunotherapeutic target in neuroblastoma

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Background

• Neuroblastoma is a solid extra cranial tumor arising from aberrant development of the sympathetic nervous system during embryogenesis.
• It is the most commonly diagnosed cancer within the first year of life with about 700 new cases diagnosed in the US each year.
• Despite current multimodal therapies, the survival rate for high-risk neuroblastoma remains lower than 50% and relapsed cases are generally incurable.

The cell surface landscape of primary and relapsed neuroblastoma (NB) is currently not well characterized. An unbiased survey of these proteins and their isoforms would greatly facilitate the identification of candidate immunotherapeutic targets for preclinical validation.

Study Approach

• Proteomic and transcriptomic data was integrated with RNA-sequencing and CNP-sequencing data to prioritize potential immunotherapeutic targets which were then validated and functionally characterized. Targets are prioritized for preclinical development.

Cell Surface Protein Isolation Method

Figure 2 Cell surface protein isolation method is highly reproducible. (A) Cell pellets or tumor samples are lysed and homogenized. Membrane proteins are isolated using ultracentrifugation. Plasma membrane proteins are further extracted using sucrose density gradient ultracentrifugation. The proteins are reduced, alkylated, digested and prepared for analysis by mass spectrometry. (B) Reproducibility between two biological replicates for all cell lines and patient derived xenografts (PDXs) analyzed (C) Identification of known cell surface proteins evaluated as immunogenic targets in NB (D) Lack of correlation between RNA (sequencing) and protein (mass spectrometry).

Validation of DLK1 cell surface expression

Figure 5 DLK1 cell surface expression validates by immunofluorescence, flow cytometry and IHC.

(A) Immunofluorescence shows colocalization of DLK1 with Cadherin in NB cell lines. (B) Flow cytometry confirms high surface expression in NB cell lines with high expression levels of DLK1, and absence in cell lines with no or low DLK1. (C) IHC staining shows preferential expression of DLK1 in NB and other pediatric malignancies compared to normal tissues.

Conclusions and Ongoing Work

• We have defined the first MS3-based surfaceome of NB.
• Identified DLK1 as an epigenetically-regulated protein and candidate immunotherapeutic target using an integrative multi-omic approach.
• ADCT-701 shows potent and specific activity in NB models with high endogenous expression of DLK1. Responses are scored as maintained complete response (CR) or progressive disease (PD).

Acknowledgements

• Alex’s Lemonade Stand Foundation (ALSF) Innovation Award “Defining the Cell Surface Landscape of Neuroblastoma: Identification of Optimal Immunotherapeutic Targets”
• W. W. Smith Medical Research Charitable Trust Grant
• F31-CA225069 and ALSF/BGS Graduate Scholars in Pediatric Cancer Research Fellowship
• Ongoing efforts include assessing how the NB surfaceome evolves under the selective pressure of chemotherapy using paired diagnostic and relapsed NB tumors and determining mechanism(s) of resistance to DLK1-directed ADC therapy.
• We will be applying our approach to other childhood cancers through a collaborative US4 in the Pediatric Immunotherapy Discovery and Development Network (P4-DDN).

ADCT-701 shows potent anti-tumor activity

Figure 7 ADCT-701 shows potent anti-tumor activity (A) DLK1 FPKM for NB PDX models. (B) IHC staining for DLK1 in panel of PDX for mouse efficacy studies. (C) Western blot showing knockdown of DLK1 in response to shRNA (C-D) DLK1 knockdown is associated with near-total outgrowth and decrease cell growth (E) Full proteome shows knockdown of DLK1 to be fourth most downregulated protein and is associated with outgrowth of neurons and neurons.

DLK1 knockdown shows a role in differentiation

Figure 6 DLK1 plays a role in differentiation (A) Images of control and DLK1 knockdown SK-N-BE2/C cells (B) Western blot showing knockdown of DLK1 in response to shRNA (C-D) DLK1 knockdown is associated with near-total outgrowth and decrease cell growth (E) Full proteome shows knockdown of DLK1 to be fourth most downregulated protein and is associated with outgrowth of neurons and neurons.

Multi-omic data integration identifies DLK1

Figure 3 Multi-omic data integration identifies DLK1. (A) Proteogenomic data integration identifies DLK1. (B) DLK1 has similar abundance to other known cell surface protein precursors. (C-D) MS on NB cell lines and RNA-Seq patient data show a subset have high levels of DLK1, while most normal tissues do not express DLK1.

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