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Targeting Gasdermin B (GSDMB) Overexpression in Cancer: Novel Compounds for HER2/GSDMB-positive Tumor Treatment

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BACKGROUND:

Gasdermin B (GSDMB) protein has a dual functionality in cancer, rendering it as a potential therapeutic target. On the one hand, this protein has the intrinsic capacity to induce pyroptosis (a proinflammatory cell death), a mechanism that could be capable of eradicating certain tumor cells. On the other hand, data from our lab revealed that GSDMB is overexpressed in 60% of HER2+ breast and gastro-esophageal tumors, where it correlates with unfavorable clinical outcomes. Moreover, our findings demonstrate that GSDMB over-expression facilitates multiple pro-tumor activities, including invasion, progression, metastasis, and resistance to anti-HER2 therapies.

OBJECTIVE:

The objective of this study is to identify and characterize novel GSDMB-targeted anti-tumor compounds, given the pivotal role of GSDMB overexpression in HER2+ cancer biological and clinical behavior.

MATERIALS AND METHODS:

Using different HER2/GSDMB+ breast cancer models, we tested the effects of various compounds on cell viability, selected through high-throughput drug screening, using different complementary in vitro approaches: Alamar Blue assay, lactate dehydrogenase (LDH) enzyme release assay, cell death measurement by flow cytometry (Annexin V/propidium iodide assay and Caspase 3/7 assays), senescent cell detection by flow cytometry using the CellEvent Senescence Green Flow kit, and cell cycle analysis by flow cytometry. Additionally, protein expression was analysed through Western blot and in silico studies were performed, including molecular docking techniques followed by Molecular Dynamics stabilization assays, to assess the potential interaction between selected compounds and the GSDMB protein. Furthermore, these findings were tested and validated in preclinical animal models using mice bearing orthotopically injected breast cancer xenografts. Lastly, RNA sequencing was conducted to elucidate the mechanisms of action of the compounds, and these findings are currently being validated by qPCR.

RESULTS:

Following a large high-throughput screening, three FDA-approved compounds (A, B and C) were initially identified as potential hits with the capacity to reduce the cell viability of GSDMB-expressing cells over GSDMB-silenced cells. Upon subsequent validation, it was confirmed that Compound B exhibited a positive GSDMB-dependent cytotoxic effect. Therefore, treatment with Compound B resulted in a preferential reduction in the viability of

GSDMB-overexpressing tumor cells. Interestingly, this compound not demonstrably influence pyroptotic cell death (as measured by LDH release), suggesting that it may eradicate GSDMB+ cells through alternative cell death mechanisms. Consequently, we tested the potential induction of apoptosis and senescence following compound B treatment. While no evidence of apoptosis has been observed, but a slightly induce senescence was observed.

Furthermore, preliminary in silico studies indicated a potential direct binding (forming a thermodynamically stable complex) of compound B with GSDMB, suggesting the possibility of a thermodynamically stable complex and reinforcing the concept of drug-target specificity. Finally, the therapeutic capacity of this compound was evaluated in preclinical in vivo mouse models using mice bearing orthotopically injected GSDMB-expressing and silencing HER2+ breast cancer cells. These results demonstrated a reduction in tumor growth and tumor weight ex vivo, as well as a higher percentage of tumor necrosis ex vivo in those tumors expressing GSDMB versus those silenced, indicating a promising therapeutic efficacy of this compound in vivo.

CONCLUSIONS:

The potential utility of compound B as a novel drug targeting GSDMB has been identified and validated at least in HER2 BC. This drug could preferentially reduce the viability of GSDMB-overexpressing breast cancer cells, and it may serve a starting point for the development of a novel and effective therapeutic approach for HER2/GSDMB+ breast cancer.

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