Identification of Asporin as a HER3 ligand exposes a therapeutic vulnerability in metastatic prostate cancer

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Abstract

Background: Rates of prostate cancer incidence and advanced-stage diagnoses are increasing. Androgen deprivation therapy (ADT) in combination with novel hormonal therapies (NHT), including apalutamide, darolutamide, enzalutamide, and abiraterone, remains the current standard of care for patients with metastatic prostate cancer, sometimes with the addition of chemotherapy. Despite initial response, nearly all patients with advanced disease progress to metastatic castration-resistant prostate cancer (mCRPC), a lethal form of the disease. There is an urgent unmet need to understand the mechanisms driving prostate cancer progression and to identify alternative therapies for patients living with mCRPC.

Methods: Asporin (ASPN)-induced HER2/HER3 activation and downstream signaling was identified by RNA-sequencing and then confirmed by immunoblotting in multiple human metastatic prostate cancer cell lines. AlphaFold2 with Rosetta refinement was used to predict the structural model of ASPN complexed with HER3 and HER2. Structural models were substantiated by co-immunoprecipitation of ASPN and HER3 in both cell-free and cell-based systems. CRISPR-cas9 targeting of HER2 and HER3 and therapeutic inhibition of HER2 with Tucatinib were used to determine the role of HER2/HER3 in ASPN-induced signaling and migration. The efficacy of Tucatinib and antibody-drug conjugates (ADC) targeting HER2 (Trastuzumab-deruxtecan) and HER3 (Patritimab-deruxtecan) were assessed in multiple metastatic prostate cancer cell lines *in vitro*. Trastuzumab-deruxtecan was assessed in PC3 xenografts *in vivo*. Prostate cancer metastases from 33 patients were assessed by IHC for HER2 and HER3 and by RNAscope for ASPN.

Results: Cancer-associated fibroblasts (CAF) are part of the tumor microenvironment that enable cancer cells to establish metastases, but the mechanisms of these interactions are not fully known. In this study, we sought to identify the molecular interactions between CAF-secreted ASPN and metastatic prostate cancer cells. We report that ASPN is a novel ligand of HER3 and induces HER3 heterodimerization with its preferred dimerization partner, HER2. ASPN activates established ErbB-associated pathways including Phosphoinositide 3-kinase (PI3K), Mitogen-activated protein kinase (MAPK), and calcium signaling, in multiple metastatic prostate cancer cell lines to promote cell migration. Genetic and molecular inhibition of HER2/HER3 mitigates ASPN-induced signaling and cell migration, suggesting these receptors are required for paracrine activation by ASPN. Importantly, small molecule and ADC therapies targeting HER2/HER3 significantly diminished prostate cancer cell growth *in vitro*, with enzalutamide-resistant cells showing increased sensitivity. Trastuzumab-deruxtecan nearly resolved tumors in an *in vivo* xenograft model. Lastly,

ASPN^{+} CAF in the TME of HER2/HER3-expressing metastatic prostate cancer is frequently observed in patient samples, supporting the clinical relevance of these findings.

Conclusions: Collectively, these findings indicate ASPN functions as a HER3 ligand to induce cellular migration, and inhibition with anti-HER2/HER3 therapies highlights potential clinical utility for patients with HER2-expressing metastatic prostate cancer.

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