Lek. Dawid Mehlich

Poszukiwanie racjonalnych strategii przełamywania chemiooporności potrójnie ujemnego raka piersi - kinaza MLK4 jako nowy cel terapeutyczny

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

Promotor: prof. dr hab. Dominika Nowis

Promotor pomocniczy: dr Anna Marusiak

Badania przeprowadzono w Laboratorium Medycyny Doświadczalnej, Centrum Nowych Technologii UW i zakończono w Laboratorium Molekularnej OnkoSygnalizacji, Międzynarodowy Instytut Mechanizmów i Maszyn Molekularnych Polskiej Akademii Nauk.

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2023 r.

Streszczenie w języku angielskim

Title: Rational strategies to overcome triple-negative breast cancer chemoresistance - MLK4 kinase as a novel therapeutic target

Breast cancer is the most often diagnosed cancer and the second leading cause of cancer-related deaths in women in Poland and worldwide. Triple-negative breast cancer (TNBC) is a subtype of breast cancer defined by the absence of estrogen receptor, progesterone receptor, and HER2 expression. TNBC is characterized by aggressive clinical behavior, rapidly progressive disease course, and poor prognosis. Over recent decades, many preclinical and clinical studies have been performed to better understand the biology of TNBC and identify more efficient therapeutic strategies. Novel therapies for TNBC have been developed, including immunotherapies, PARP inhibitors, and antibody-drug conjugates. Despite these major advances, treatment of TNBC is still an unmet need. Due to the lack of routine targeted therapies, non-selective chemotherapy remains the most used therapeutic option for TNBC patients. Nevertheless, both intrinsic and acquired chemoresistance limits the clinical efficacy of the current therapies, leading to high relapse rates and poor prognosis. Therefore, there is a need for basic science research aiming to identify the molecular mechanisms of chemoresistance, which may translate into novel treatment strategies. Protein kinases are enzymes that alter the activity of their substrates through phosphorylation (transferring of the phosphate molecule). They play a crucial role in most signaling cascades, thereby regulating numerous processes within the cell. Aberrant activation of various protein kinases has been implicated in the pathogenesis of different types of human cancers, including TNBC. Small molecule inhibitors targeting protein kinases have been tested in clinical trials in patients with TNBC. However, most of the trials evaluating kinase inhibitors, either in monotherapy or in combination with chemotherapy, demonstrated only limited clinical efficacy of these agents.

The first publication included in this series is a review article describing the past and ongoing preclinical and clinical studies that evaluated the therapeutic efficacy of small-molecule kinase inhibitors in TNBC therapy. In this article, I described that TNBC heterogeneity significantly affects tumor response to targeted therapies. Furthermore, by performing a comprehensive literature review and analyzing ongoing studies, I demonstrated that the majority of the clinical trials focused only on a small subset of well-characterized kinases. Considering these observations, identifying novel druggable kinases will likely expand the therapeutic landscape of TNBC and provide new therapeutic solutions for TNBC patients.

In the second article included in this series, I described the original studies that characterized the role of MLK4 kinase in TNBC chemoresistance. Mixed-Lineage Kinase 4

(MLK4) is a member of the MAP3K family of serine/threonine kinases. High-throughput sequencing data indicated that the MLK4 gene (*MAP3K21/KIAA1804*) is amplified and/or overexpressed in 20% of breast cancer cases and over 50% of TNBC cases. Our previous studies demonstrated that MLK4 promotes breast cancer cell lines proliferation and aggressive growth *in vitro* and *in vivo*. Here, I aimed to investigate the role of MLK4 kinase in TNBC chemoresistance. By analyzing gene expression database (GEO NCBI), I found that high MLK4 expression in tumor tissue is associated with poor survival of TNBC patients treated with neoadjuvant chemotherapy. Next, using TNBC cell line models, I demonstrated that MLK4 promotes chemoresistance and survival of human TNBC cells *in vitro* and in mouse xenograft models *in vivo.* MLK4 knock-down or inhibition sensitized TNBC cell lines to several clinically used chemotherapeutic agents. I observed that MLK4-deficient cells displayed enhanced apoptosis induction and persistent DNA damage accumulation upon treatment with chemotherapeutics using flow cytometry and confocal microscopy techniques. To further investigate the mechanisms of MLK4-dependent chemoresistance of TNBC cells, I used phosphoproteomic profiling, reporter assays, and CRISPR/Cas9 MLK4 knock-out cells. I found that MLK4 regulates DNA damage repair in response to genotoxic chemotherapy. Mechanistically, I demonstrated that loss of MLK4 impairs activation of the ATM kinase, which plays a major role in coordinating DNA damage response. Furthermore, I performed RNA-seq analysis of MLK4-depleted and control cells treated with chemotherapy, which indicated that MLK4 is required for DNA damage-induced activation of ATM-NEMO signaling axis and expression of several NF-κB dependent genes that facilitate TNBC chemoresistance.

In summary, protein kinases may serve as attractive therapeutic targets in the treatment of TNBC and overcoming tumor chemoresistance. Future preclinical and clinical studies investigating kinase inhibitors in TNBC should account for tumor heterogeneity that significantly affects the response to targeted therapies. Moreover, the identification of novel kinase targets is warranted, as it may significantly broaden the therapeutic landscape of TNBC. Here, I demonstrated for the first time that MLK4 confers chemoresistance in TNBC, and thus it may serve as a new therapeutic target in TNBC treatment. These results suggest that small-molecule MLK4 inhibitors combined with chemotherapy might be used as a rational treatment strategy for many TNBC patients. Moreover, I discovered novel and noncanonical functions of MLK4 kinase in regulating DNA damage response signaling.