ID: 03108

Topic: DRUG TOLERANCE

Unmasking cancer cells to maximize impact of HER2-targeting antibody-drug conjugates

Maria Bordoy[1,2] , Denisse Wolf[3] , Laura J. Van't Veer[3] , John Martens[2] , Roos Vincken[1,4] , Ana Ruiz-Saenz[1,2,4]

1) Department of Cell Biology, Erasmus University Medical Center Rotterdam, 3015 GD, Rotterdam, The Netherlands 2) Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, 3015 GD Rotterdam, The Netherlands 3) Department of Laboratory Medicine, University of California, San Francisco, 2340 Sutter Street, San Francisco, CA 94143, USA 4) Cancer Therapy Resistance lab, Bizkaia Science and Technology Park, building 800, Derio, Bizkaia

Introduction:

Aberrant glycosylation of proteins is a hallmark of cancer and has been linked to multiple cellular processes, including receptor activation, protein localization, and interaction. The tyrosine kinase receptor HER2, which is the key driver of HER2-positive breast cancers, is heavily glycosylated and is the target for current antibody-based treatments. In the last years, new therapies, known as antibody-drug conjugates (ADCs), have been developed by combining the monoclonal antibody anti-HER2 trastuzumab, with different cytotoxic payloads. Two of these ADCs, T-DM1 and T-DXd are key elements of the armamentarium for the treatment of HER2-positive breast cancers. Despite the clinical success of ADCs, resistance to these therapeutic agents remains a significant challenge. While most studies addressing potential mechanisms of resistance have focused on intracellular signalling pathways, cancer cell glycosylation has been largely overlooked.

Objectives:

In this scenario, we aim to determine the impact of aberrant glycosylation of breast cancer cells on the response to HER2-targeting agents. We seek to identify specific glycogenes associated with the efficacy of T-DM1 in HER2-positive breast cancer and to explore the role of altered glycosylation patterns on ADC binding and internalization.

Methods:

A set of 126 glycosylation-related genes was analysed for their association with the response to HER2-targeting agents using patient data from the I-SPY2 clinical trial. To validate these findings, siRNA-mediated gene silencing was employed to knock-down specific glycogenes in different cell models of HER2-positive breast cancer. The effectiveness of siRNA knockdowns was confirmed through quantitative PCR and Western blotting. Additionally, lectin-based flow cytometry was used to assess changes in cell surface glycosylation following gene knockdowns. Cell viability assays were then conducted to evaluate the impact of altering glycosylation on the response to T-DM1. Furthermore, different N-glycosylation sites on the HER2 receptor were characterized by generating HER2 glycosylation mutants using site-directed mutagenesis to assess their impact on T-DM1 sensitivity.

Results:

Bioinformatics analysis of clinical data revealed a strong correlation between the expression of specific glycogenes and the response to T-DM1 in HER2-positive breast cancer patients. Specifically, tumours with elevated expression of a particular N-acetylglucosamine and fucosyl transferase show significantly higher sensitivity to T-DM1, suggesting their potential role as predictive biomarkers. *In vitro* validation through loss-of-function experiments in HER2-positive breast cancer cell lines further confirmed these findings, showing that knocking-down the N-acetylglucosamine transferase and fucosyltransferase resulted in increased resistance to the ADC, T-DM1. Moreover, the study of N-glycosylation sites on the HER2 receptor revealed that mutations in those N-glycosylation sites closer to the trastuzumab binding site, substantially impacted T-DM1 response. Specifically, N530Q, N571Q, and N629Q mutants exhibited increased sensitivity to T-DM1.

Conclusions:

Our results suggest that cell surface glycosylation influences the sensitivity to the antibody-drug conjugate, T-DM1. These findings have fuelled additional studies in our group using other trastuzumab-based ADCs such as the recently developed T-DXd, that promises to revolutionized the treatment of breast cancer patients. Understanding of the role of glycosylation in response to antibody-based targeting treatments could lead to the identification of glycan-based biomarkers for response to HER2-targeting ADCs and open potential avenues of commercialization in the form of antibodies against tumour-specific glycan-based antigens.