Identification of potential novel drivers and biomarkers of CDK4/6 inhibitor resistance in metastatic breast cancer

Leonie Florence Ott[1]
Malik Alawi[2], Lia Kracht[1], Malgorzata Stoupiec[1], Klaus Pantel[1] and Sabine Riethdorf[1]
[1] Institute of Tumor Biology, Center of Experimental Medicine, University Medical Center Hamburg- Eppendorf, Hamburg, Germany
[2] Bioinformatics Core, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background & objectives

Background

Inhibitors of the cyclin dependent kinases 4 and 6 (CDK4/6i) have become part of the standard of care for patients with metastatic breast cancer. Despite their initial benefit for most patients, the development of resistance to this treatment is almost inevitable. However, predictive biomarkers are hardly described, and their definition remains an unmet challenge.

Objectives

We aimed to identify novel drivers and potential biomarkers of CDK4/6i resistance by establishing and characterizing two ribociclib-resistant breast cancer cell lines. Additionally, we investigated whether microRNAs contribute to the development of resistance. This class of non-coding RNAs are important regulators of post-transcriptional gene expression but are poorly characterized in the context of CDK4/6i resistance.

Methods

Ribociclib-resistant derivatives of MCF7 and CTC-ITB-01 cell lines were created by long term exposure to the inhibitor for a minimum of 6 months and potential novel resistance drivers were identified by transcriptome analysis using RNA-sequencing. Resistance was confirmed by functional assays such CCK8-assay, colony formation assay and cell cycle analysis. In addition to mRNAs encoding proteins that may contribute to resistance, we also investigated microRNAs. Altered expression levels were confirmed by either mRNA or microRNA qPCR. Additionally, protein levels were determined by Western blot analysis and microRNA levels were evaluated by microRNA *in-situ* hybridization.

Results

The RNA-sequencing analysis identified ≥ 2000 genes with dysregulated expression in both resistant cell lines compared to their respective parental counterparts. Overrepresentation

analysis revealed that a high number of these genes encode proteins associated with the cell cycle and/or epithelial-to-mesenchymal transition (EMT). We found increased expression of CDK14 in the resistant versus parental CTC-ITB-01 cell line and selected this protein for further experiments, as CDK14 is not only a cell cycle driver but also associated with EMT and stemness in breast cancer. Furthermore, we found that the levels of miR-205-5p, a microRNA often described as a negative regulator of EMT, were decreased in the same cell line. Since CDK14 is a predicted target of miR-205-5p, we hypothesize a potential regulatory axis that may contribute to CDK4/6i resistance.

Conclusion

Through our comprehensive transcriptome analysis of two ribociclib-resistant cell lines, we identified several novel potential resistance drivers. Their utility as predictive biomarkers requires further investigation.

Acknowledgment: This project received fundings from the Deutsche Krebshilfe DETECT high project (Nr.: 70112504) and the Horizon 2020 program RNADiagon (Nr.: 824036).