Abstract

Background: Dysregulation of cell cycle commonly occurs in cancer including triple negative breast cancer (TNBC). Recently, some pharmacological inhibitors that targeted the cell cycle checkpoints were approved by the FDA to treat the advanced metastatic HR+/HER2- breast cancer patients, but not the TNBC patients. The cyclin-dependent kinases are the main machinery to control the cell cycle. The CDK expression and activation are tightly regulated by particular mechanisms such as the Wnt signaling pathway and some microRNAs. By understanding the axis of CDK/Wnt/microRNA, we hope to improve basic knowledge of cell cycle regulation in the TNBC and to uncover the possibility of a new approach to treat TNBC patients. Here we provided the evidence to support the importance of CDK/Wnt/microRNA in the TNBC. In addition, we found a potential small compound (4AAQB) that can inhibits cell cycle through CDK 2/4 in the TNBC cells.

Methods: To investigate the best candidate of miRs that upregulated in the TNBC patients, we performed Nanostring assay and bioinformatics analysis using Indonesian TNBC patients and open source datasets. To explore the mechanism of CDK/Wnt axis, we performed in silico, and in vitro studies. Firstly, to confirm the importance of CDK 2/4/6 in breast cancer patients, we used 5 datasets that were downloaded from cBioportal. To test the importance of CDK2/4 in carcinogenesis of TNBC, we performed in vitro. After generated the low expression of CDK 2/4 clone cells, we performed western blot, cell count, migration invasion, and tumorsphere assays. To explore the potential anti-cancer activity of the 4-AAQB, we treated the MDA-MB-231 and Hs578t cell lines with several doses of 4-AAQB. Further, we performed functional assays such as SRB, western blot, annexin V, immunofluorescent, and migration assays.

Results: We found 10 upregulated miRs in the primary tumor tissue of TNBC patients using the Nanostring assay. We confirmed that hsa-miR-200c-3p and hsa-miR-141-3p were upregulated in TNBC tissue, serum samples and correlated with the poor prognosis of patients. In silico analysis revealed that the cell cycle pathway was the most affected pathway by 10 upregulated miRs. Further, our results showed the CDK 2/4 upregulation have a significant correlation to the breast cancer patients overall survival, but not the CDK 6. In vitro study show that the CDK 2/4 inhibition through Wnt signaling pathway suppression disrupted the cell proliferation, migration, invasion, and the tumorigenicity of TNBC cells. The 4-AAQB treatment inhibited the CDK 2/4 expression of TNBC cells in a dose-dependent manner. The functional assays showed that the 4-AAQB could suppress the aggressiveness of TNBC cells. The CDK 2/4 inhibition with 4-AAQB induced cell proliferation inhibition, apoptosis, and DNA damages.

Conclusion: Based on our results, we provide the basic knowledge of the miRs/Wnt/CDK axis as an important regulator in the cell proliferation in TNBC. Further, we provided compelling evidence of the potential therapeutic effects of a natural small compound, 4-AAQB by targeting the CDK2/4 in TNBC cells.

Keywords: Breast cancer, TNBC, microRNA, Wnt, CDK 2/4, 4-AAQB.