Elevation of Histone deacetylase-3 and -7 mediates lapatinib-induced cell migration and invasion of triple-negative breast cancer cells through COX-2 overexpression

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Running Title: Elevation of HDAC by lapatinib confers metastasis of triple-negative cells

Keywords: Breast cancer, HDAC, lapatinib, COX-2, metastasis

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Abstract

Lapatinib, a dual EGFR/HER2 tyrosine kinase inhibitor, has been shown to improve the survival rate of patients with advanced HER2-positive breast cancers. In attempt to broaden its clinical use, lapatinib was tested in combination with chemotherapy in HER2-negative diseases according to its activity against EGFR, but unfortunately has been shown to worsen overall survival of breast cancer patients with triple-negative (TN) or HER2-negative/PgR-negative tumors. Our previous study also explored that treatment with lapatinib renders TN cells more metastatic via increasing COX-2 and EGFR expressions, providing a possible explanation for the clinical observation. However, the molecular mechanism underlying the induction of COX-2 and EGFR expressions by lapatinib remains unclear. Here, we showed that up-regulation of histone deacetylases (HDAC) 3 and 7, accompanying with the deacetylation of histone H3K9 and H2BK5, were found in the lapatinib-treated TN cells. Treatment with HDAC inhibitors (SAHA and TSA) or HDAC3 and HDAC7 siRNA dramatically reduced lapatinib-mediated cell migration and invasion through down-regulation of COX-2 expression transcriptionally. Both activations of AP-1 and NF-kB were observed and mediated the COX-2 gene expression in the lapatinib-treated TN cells, HDAC3/7 inhibition inhibited AP-1 but not NF-kB activation. Interestingly, treatment

with TSA, but not SAHA and HDAC3/7 siRNA, attenuated the expression of EGFR via up-regulating miR-7 expression which can target the 3'-UTR of EGFR mRNA, suggesting that inhibitions of HDAC3 and HDAC7 by TSA and SAHA more specifically reduced COX-2 transcription in a AP-1-dependent but NF-kB-independent manner and that inhibitions of other HDACs by TSA may contribute to miR-7 expression and the subsequent EGFR down-regulation. Unexpectedly, our data further showed that lapatinib-treated TN cells are more sensitive to TSA or SAHA than their parental cells. Moreover, silence of HDAC7, but not HDAC3, expression dramatically reduced the viability of lapatinib-treated TN cells through induction of apoptosis, suggesting that targeting HDAC7 may be crucial for the antitumor activity of HDAC inhibitor. Together, our results revealed that alterations of HDAC3/7 expression are involved in lapatinib-induced COX-2 expression and migration of triple-negative cells, and that co-treatment with HDAC inhibitors may show benefits for patients who received lapatinib therapy.