The molecular targets and anti-invasive effects of 2,6-bis-(4-hydroxyl-3methoxybenzylidine) cyclohexanone or BHMC in MDA-MB-231 human breast cancer cells

ABSTRACT

In order to metastasize, tumor cells need to migrate and invade the surrounding tissues. It is important to identify compound(s) capable of disrupting the metastasis of invasive cancer cells, especially for hindering invadopodia formation, so as to provide anti-metastasis targeted therapy. Invadopodia are thought to be specialized actin-rich protrusions formed by highly invasive cancer cells to degrade the extracellular matrix (ECM). A curcuminoid analogue known as 2,6-bis-(4-hydroxy-3-methoxybenzylidine)cyclohexanone or BHMC has shown good potential in inhibiting inflammation and hyperalgesia. It also possesses an anti-tumor effects on 4T1 murine breast cancer cells in vivo. However, there is still a lack of empirical evidence on how BHMC works in preventing human breast cancer invasion. In this study, we investigated the effect of BHMC on MDA-MB-231 breast cancer cells and its underlying mechanism of action to prevent breast cancer invasion, especially during the formation of invadopodia. All MDA-MB-231 cells, which were exposed to the non-cytotoxic concentrations of BHMC, expressed the proliferating cell nuclear antigen (PCNA), which indicate that the anti-proliferative effects of BHMC did not interfere in the subsequent experiments. By using a scratch migration assay, transwell migration and invasion assays, we determined that BHMC reduces the percentage of migration and invasion of MDA-MB-231 cells. The gelatin degradation assay showed that BHMC reduced the number of cells with invadopodia. Analysis of the proteins involved in the invasion showed that there is a significant reduction in the expressions of Rho guanine nucleotide exchange factor 7 (β-PIX), matrix metalloproteinase-9 (MMP-9), and membrane type 1 matrix metalloproteinase (MT1-MMP) in the presence of BHMC treatment at 12.5 µM. Therefore, it can be postulated that BHMC at 12.5 µM is the optimal concentration for preventing breast cancer invasion.

Keyword: BHMC; Breast cancer invasion; Invadopodia; β-PIX; MMP-9; MT1-MMP