論　文　要　約

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Identification of Hirsutine as a novel anti-cancer phytochemical and exploration of its anti-tumor mechanism against breast cancer

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Introduction

Breast cancer is the most frequently diagnosed cancer and is therefore the leading cause of cancer death in women worldwide. Despite the advances in the treatment of breast cancer, there is still a high mortality rate in breast cancer patients; therefore the development of new agents for breast cancer is clinically important.

Phytochemicals from natural products are a promising source for the development of novel cancer therapeutics. Because of their potential effectiveness and low toxicity profiles.

Nuclear factor-κB (NF-κB), defined as a multi-functional transcription factor, plays a critical role in the initiation, promotion and progression of certain types of cancers through its ability to up-regulate genes responsible for cell survival, invasion, angiogenesis, and metastasis. Consequently, the NF-κB pathway is regarded as a potential new drug target in cancer metastasis and progression. In this study, we screened 56 phytochemical compounds for their inhibitory activity in NF-κB. Hirsutine was found to be a prominent NF-κB inhibitor and showed potential anti-metastatic effect. We also explored the anti-tumor activity of hirsutine in human breast cancer cells and found as a promising compound for breast cancer therapy by inducing DNA damage response.

1. Identification of hirsutine as an anti-metastatic phytochemical by targeting NF-κB activation

Nuclear factor-κB (NF-κB) activation has been implicated not only in carcinogenesis but also in cancer cell invasion and metastatic process; therefore, targeting the NF-κB pathway is an attractive strategy for controlling

metastasis. Amongst 56 chemically defined compounds derived from natural products, we have identified a new phytochemical compound hirsutine, which strongly suppress NF-κB activity in murine 4T1 breast cancer cells. In accordance with their NF-κB inhibition, hirsutine reduced the metastatic potential of 4T1 cells, as seen in the inhibition of the migration and invasion capacity of 4T1 cells. Hirsutine further inhibited the constitutive expression of MMP-2 and MMP-9 in 4T1 cells reduced the in vivo lung metastatic potential of 4T1 cells in the experimental model. Given that the migration of human breast cancer cells was also inhibited, our present study implies that hirsutine is an attractive phytochemical compound for reducing metastasis potential of cancer cells in accordance with regulating tumor-promoting NF-κB activity.

2. Selective anti-cancer activity of hirsutine against HER2 positive breast cancer cells by inducing DNA damage

To further examine the clinical utility of hirsutine, we investigated the anti-tumor activity of hirsutine on human breast cancer cells. Among six distinct human breast cancer cell lines, hirsutine showed strong cytotoxicity against HER2 positive/p53 mutated MDA-MB-453 and BT474 cell lines. Conversely, HER2 negative/p53 wild-type MCF-7 and ZR-75-1 cell lines showed resistance against hirsutine-induced cytotoxicity. Hirsutine induced apoptotic cell death in MDA-MB-453 cells, but not in MCF-7 cells, through activating caspases. Furthermore, hirsutine induced DNA damage response in MDA-MB-453 cells, but not in MCF-7 cells, as highlighted in the up-regulation of γH2A.X expression. Along with the induction of DNA damage response, the suppression of HER2, NF-κB and Akt pathways and the activation of p38 MAPK pathway in MDA-MB-453 cells were observed. Considering that there was no difference between MDA-MB-453 and MCF-7 cells in irinotecan-induced DNA damage response, our presented results indicate selective anti-cancer activity of hirsutine in HER2 positive breast cancer by inducing DNA damage response.

3. Targeting ATM pathway for effective therapy against hirsutine-resistant breast cancer cells

For establishing effective therapy against breast cancer by using hirsutine, we next explored the mechanism underlies by which protect hirsutine-resistant

breast cancer cell lines, MCF-7 and ZR-75-1 cell lines. To identify potential targets for overcoming such hirsutine-resistance, we tested chemical inhibitors for their reversal effect on hirsutine-resistance in MCF-7 cells and found that the inhibition of ATM sensitized MCF-7 cells to hirsutine-induced cell death. In accordance with the sensitization to hirsutine, the up-regulation of γH2A.X expression was observed upon the co-treatment with hirsutine and ATM inhibitor, KU-60019. While the co-treatment with hirsutine and KU-60019 induced cell death in MCF-7 cells expressing dominant negative p53, the ROS expression was significantly increased upon the co-treatment. Taken together, these findings suggest that the inhibition of ATM pathway enhances anti-cancer effect of hirsutine possibly through the induction of p53-independent, ROS-mediated DNA damage pathway.

Conclusion

In this study, hirsutine was found to be a prominent NF-κB inhibitor and significantly inhibited the metastatic potential of murine 4T1 breast cancer cells both in vitro and in vivo. The investigation on six human breast cancer cells indicated selective anti-cancer activity of hirsutine in HER2 positive breast cancer by inducing DNA damage response. In addition, the resistance of breast cancer against hirsutine treatment can be reversed by targeting ATM pathway possibly through an induction of ROS expression. Collectively, the present study will provide important pre-clinical evidences for establishing a novel breast cancer therapy by using hirsutine.