

Changing redox state potentiates all-trans retinoic acid cytotoxicity against CD44⁺ and CD117⁺ malignant melanoma cells

Abstract

Background: Malignant melanoma and treatment failure are, unfortunately, two intertwined clinical problems with a profound social impact. A simple look at the statistics is alarming: the lifetime risk of melanoma continues to increase steadily, reaching 1 in 75. All-trans retinoic acid (ATRA) is a differentiating agent that inhibits tumor cell growth. Despite their potent antitumor properties, some melanoma cell lines are highly resistant to the ATRA treatment. Here, we hypothesized that NAC and allicin can sensitize malignant melanoma cells to ATRA.

Material and methods: To clarify this mechanism, we determined the sensitivity to ATRA, allicin, NAC/ATRA, allicin/ATRA in two CD44⁺ and CD117⁺ melanoma cell subpopulations. The CD44⁺ and CD117⁺ cells were sorted from the A375 cell line by using the magnetic-activated cell sorting (MACS). The anticancer effects were examined by cell proliferation MTT assay. Additionally, flow cytometry was used to detect cell cycle arrest. Moreover, mRNA levels of cyclin D1, MMP9, RAR β and caspase-3 gene expression were measured using real-time PCR.

Result: The CD44⁺ melanoma cells were more resistant than CD117⁺ cells to allicin or ATRA alone treatments. Importantly, the combination treatment with NAC and allicin significantly reduced the IC₅₀ values obtained for ATRA alone in CD44⁺ melanoma cells by causing cell cycle arrest at different phases. IC₅₀ value for ATRA alone was 37.43 \pm 0.54, while IC₅₀ values for NAC/ATRA and allicin/ATRA treatments were 11.72 \pm 0.22 μ M 17.53 \pm 0.2 μ M, respectively. Additionally, ATRA, NAC/ATRA, and allicin/ATRA induced a significant increase of cyclin D1 mRNA level in CD44⁺ and CD117⁺ cells. Furthermore, allicin alone resulted in a remarkable

reduction of MMP-9 mRNA expression in both CD44⁺ and CD117⁺ cells. In contrast, ATRA and the combination treatments increased MMP-9 mRNA expression in CD44⁺ cells.

Conclusion: *our results indicate that NAC and allicin reinforce the ATRA-mediated inhibitory effects on CD44⁺ and CD117⁺ cells and may provide a new approach for the treatment of malignant melanoma.*

Keywords: *all-trans retinoic acid, melanoma, allicin, CD44, CD117, reactive oxygen species (ROS)*