Arsenic trioxide-mediated Notch pathway inhibition depletes the cancer stem-like cell population in gliomas

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Abstract

Cancer stem-like cells (CSCs) are potential targets for treatment of glioblastoma multiforme (GBM) due to their role in tumorigenesis and recurrence. In this study, we investigated the inhibitory effect of arsenic trioxide (As2O3) on CSCs of GBM in human glioma cell lines (U87MG, U251MG and U373MG) in vivo and in vitro. Immunofluorescence staining and flow cytometry revealed that the percentage of Nestin-positive cells in the aforementioned cell lines was diminished by 12%, 14% and 7%, respectively, after treatment with 2 μM As2O3. Furthermore, we used soft-agar in U87MG and tumor xenografts in nude mice to demonstrate the ability of As2O3 to inhibit the formation of tumor in the three cell lines. These results indicate the negative regulation of CSCs by As2O3. In addition, a Western blot analysis revealed decreased levels of Notch1 and Hes1 proteins due to As2O3 treatment. We conclude that As2O3 has a remarkable inhibitory effect on CSCs in glioma cell lines in vivo and in vitro; in addition, we determined that the mechanism of CSC inhibition involves the deregulation of Notch activation.

1. Introduction

GBM is notorious for its resistance to treatment and high frequency of recurrence. However, the potential to understand the mechanisms of treatment resistance and recurrence has increased with the identification of rare populations of cancer stem-like cells in GBM [1,2]. A novel GBM combined chemo- and radiotherapy strategy that targets CSCs has received extensive attention. Despite recent reports of CSC resistance to chemo- and radiotherapy [3,4], some agents which regulate signaling pathways have been shown to inhibit CSC growth in GBM. For example, cyclopamine, which blocks the Hedgehog pathway, can reduce the CSC population in GBM [5].

Because CSCs share common properties with normal stem cells, it is plausible that they have overlapping regulation mechanisms. Indeed, one of the most pressing questions concerning the biology of stem cells involves the mechanism of how stem cells maintain self-renewal properties and continue to proliferate. Several studies have demonstrated a key role for genes associated with chromatin regulation and the cell cycle [6–8]. Meanwhile, there is significant evidence that the deregulation of some pathways inhibits tumorigenesis. CSC signaling pathways, such as the Notch signaling pathway, may be required for the survival and growth of CSCs in GBM [9]. Notch promotes the survival and proliferation of non-neoplastic neural stem cells and inhibits their differentiation [10,11]. Notch1 is an important transmembrane receptor in the family of Notch receptors. Notch signaling is initiated by ligand binding, which is followed by intramembranous proteolytic cleavage of the Notch1 receptor to generate a Notch intracellular domain (NICD). The NICD subsequently translocates to the nucleus to act as a transcriptional activator [12]. The proliferation of CSCs could be inhibited by a blockade of the Notch pathway due to the deletion of...
attributed to the deregulation of the Notch signaling activity.

The evidence that a relatively low concentration of As$_2$O$_3$ effectively inhibits proliferation of U87MG, U251MG and U373MG cells in vitro and in vivo suggests that this drug may be considered for testing on animal models and possibly for clinical trials on glioma patients. Moreover, this study is the first to address the mechanism of As$_2$O$_3$-induced suppression of CSLCs in glioma cell lines. Further study will determine the existence of other mechanisms, such as the interaction of Notch signaling with multiple other pathways and As$_2$O$_3$-induced autophagy. In summary, we demonstrated that Notch pathway blockade by As$_2$O$_3$ in glioma cell lines depletes the population of CSLCs, which are required for in vivo tumor formation. As$_2$O$_3$ thus suppresses tumorigenesis in those cell lines. This conclusion provides a foundation for further experimental studies concerning the use of As$_2$O$_3$ in the clinical therapy of GBM.

Conflict of interest

There are no conflicts of interest.

Acknowledgements

The authors thank Akira Saitou for his expert technical assistance and Brain Quinn for his help in the preparation of the manuscript. This work was supported by the grant from The National Natural Science Foundation, grant-in-aid for scientific research of China 30772239. Yunbo Zhen and Shiguang Zhao gratefully acknowledged to this work.

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