**REV3L** confers chemoresistance to cisplatin in human gliomas: The potential of its RNAi for synergistic therapy

Huibo Wang, Shu-Yu Zhang, Shuai Wang, Juan Lu, Wenting Wu, Lin Weng, Dan Chen, Yu Zhang, Zhipeng Lu, Jingmin Yang, Yuanyuan Chen, Xu Zhang, Xiaofeng Chen, Caikun Xi, Daru Lu, and Shiguang Zhao

Department of Neurological Surgery, Brain Tumor Research Center, First Affiliated Hospital, Harbin Medical University, Harbin (H.W., X.Z., X.C., S.Z.); State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai (H.W., S.-Y.Z., J.L., W.W., D.C., Y.Z., Z.L., J.Y., Y.C., D.L.); State Key Laboratory of Medical Genomics and Shanghai Institute of Hematology, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai (S.W., L.W.); Department of Neurological Surgery, Shanghai 6th People Hospital, Shanghai Jiao Tong University, Shanghai (C.X.); China

The REV3L gene, encoding the catalytic subunit of human polymerase ζ, plays a significant role in the cytotoxicity, mutagenicity, and chemoresistance of certain tumors. However, the role of REV3L in regulating the sensitivity of glioma cells to chemotherapy remains unknown. In this study, we investigated the expression of the REV3L gene in 10 normal brain specimens and 30 human glioma specimens and examined the value of REV3L as a potential modulator of cellular response to various DNA-damaging agents. Reverse transcriptase PCR/real-time PCR analysis revealed that REV3L was overexpressed in human gliomas compared with normal brain tissues. A glioma cell model with stable overexpression of REV3L was used to probe the role of REV3L in cisplatin treatment; upregulation of REV3L confers chemoresistance to cisplatin in human gliomas: The potential of its RNAi for synergistic therapy markedly attenuated cisplatin-induced apoptosis of the mitochondrial apoptotic pathway. We therefore assessed the REV3L-targeted treatment modality that combines suppression of REV3L expression using RNA interference (RNAi) with the cytotoxic effects of DNA-damaging agents. Downregulation of REV3L expression significantly enhanced the sensitivity of glioma cells to cisplatin, as evidenced by the increased apoptosis rate and marked alterations in the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xl) and proapoptotic Bcl-2-associated x protein (Bax) expression levels, and reduced mutation frequencies in surviving glioma cells. These results suggest that REV3L may potentially contribute to gliomagenesis and play a crucial role in regulating cellular response to the DNA cross-linking agent cisplatin. Our findings indicate that RNAi targeting REV3L combined with chemotherapy has synergistic therapeutic effects on glioma cells, which warrants further investigation as an effective novel therapeutic regimen for patients with this malignancy. Neuro-Oncology 11, 790–802, 2009 (Posted to Neuro-Oncology [serial online], Doc. D08-00174, March 16, 2009. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2009-015)

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the mitochondrial apoptotic pathway in glioma cells. These results are clearly reminiscent of those of REV7L knockdown, which was previously reported to confer hypersensitivity to certain types of chemotherapeutic agents, especially cisplatin, through activation of apoptosis. Although the redundant functions of specialized DNA polymerase family members in TLS remain elusive, it seems that REV3L may play a predominant role in regulating the sensitivity to the DNA cross-linking agent cisplatin in human gliomas. RNAi is emerging as a powerful approach for gene target therapy. With recent advances in RNAi delivery strategy, the shRNA complex could be efficiently delivered to the brain. Therefore, downregulation of REV3L expression by RNAi in combination with cisplatin could enhance the clinical efficacy of chemotherapy for glioma patients. In summary, we have shown for the first time that REV3L is overexpressed in human gliomas, especially in high-grade gliomas. Enhancement of REV3L expression in glioma cells resulted in reduced sensitivity to cisplatin-induced cell death. Inhibition of Bcl-2 in REV3L-overexpressing cells by HA14-1 significantly promoted cisplatin-induced apoptosis. Furthermore, suppression of REV3L expression by RNAi enhanced the sensitivity of glioma cells to cisplatin, led to more pronounced apoptosis in association with marked alterations in Bcl-2, Bcl-xl, and Bax expression levels, and reduced frequencies of cisplatin-induced mutations in glioma cells. Future studies are therefore warranted to determine the function of REV3L in vivo, especially its role in tumorigenesis and chemoresistance. Whether other DNA polymerases involved in TLS might be responsible for chemoresistance to DNA-damaging agents in human malignancies remains to be examined.

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References