UBE2D3 gene overexpression increases radiosensitivity of EC109 esophageal cancer cells

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Radiotherapy is widely used in adjuvant approaches for esophageal cancer (EsC) to reduce local recurrence and improve survival. However, the overall 5-year survival was about 17% over the past few decades. Therefore, find an effective way to improve the effect of radiotherapy is vital. We have been engaged in this work for 15 years. Not only radiosensitivity is associated with a collection of associated proteins and telomere, we found, but also telomerase. Telomerase is, furthermore, regulated by post-translational modifications of the rate limiting catalytic subunit hTERT. We had used yeast two-hybrid method to identify UBE2D3 encoding an E2 ligase which is a principle hTERT-interacting protein and inhibition of UBE2D3 expression attenuates radiosensitivity of MCF-7 human breast cancer cells by increasing hTERT expression and activity and we found that UBE2D3 is negatively correlated with hTERT expression and is a positive prognostic factor in EsC. To verify a possible contribution of UBE2D3 to tumor cell radiosensitivity, esophageal squamous carcinoma cells (EC109 cells) were transfected with the expression plasmid encoding UBE2D3 and stable transfectants were subsequently established. UBE2D3-overexpressing cells exhibited an increased incorporation of radiosensitivity, to further investigate the mechanism; the CCK-8 assay was used to confirm cell proliferation, which showed that UBE2D3 downgrades EC109 cells propagation. Moreover, cell cycle distribution was examined by flow cytometry, UBE2D3 overexpression in EC109 cells causes prolonged G1 arrest after IR exposure on the contrary G2/M shortened. We, then, detected the protein expression about ATM/ATR-Chk2 pathway by western blotting, which in UBE2D3 over-expressing cells showed decrease after irradiation. And overexpression of UBE2D3 decreases the protein level of hTERT relative to the control cell line. Subsequently, we immunoprecipitated with anti-hTERT antibody followed by immunoblotting with anti-ubiquitin antibody to examine the in vivo role of UBE2D3 in ubiquitination of hTERT. Overexpression of UBE2D3 caused a clear and dramatic increase in the amount of ubiquitinated hTERT species after 2 hours of specific proteasome inhibitor MG132 treatment, which points out hTERT may be degraded by the proteasome pathway. To determine whether this change can influence the telomere length, we used real-time PCR to test the relative telomere length and result suggests that overexpressed UBE2D3 is negative correlation with telomere extension. In conclusion, these findings suggest that UBE2D3 may be a potential target in the radiotherapy of EsC.

Biography

Xiaojia Gao has completed his Bachelor's degree from Hubei University of Traditional Chinese Medicine in 2013. He is currently pursuing his Master's degree in radiation-guided gene therapy of cancer at Wuhan University, China. He has published 2 papers in national journals and presented results of his research at a national conference.

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