RAD6 inhibition attenuates DNA repair and stem cell signaling and overcomes acquired chemoresistance in ovarian cancer

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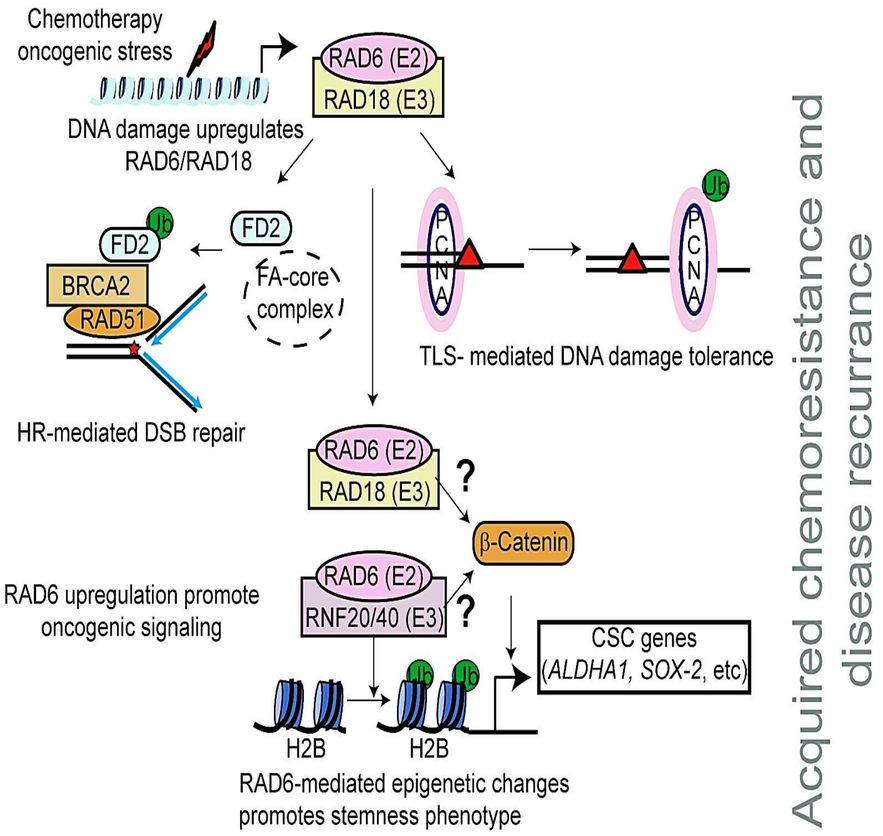
Abstract

Ovarian cancer (OC) is fifth leading cause of cancer related deaths in women. However, ~70% of OC patients relapse and develop secondary resistance to therapy. Studies show that RAD6, an E2 ubiquitin-conjugating enzyme, is significantly overexpressed in ovarian tumors. Our data shows that RAD6 overexpression leads to an increase in DNA damage response proteins such as FANCD2, PCNA, RAD18, and γH2AX after carboplatin therapy. RAD6 up-regulation also promotes increased expression of cancer stem cell signaling proteins ALDH1A1 and SOX2 in OC cell lines, which contributes to cancer recurrence and chemoresistance. Down-regulation of RAD6 using siRNA or small molecule inhibitor TZ9 attenuated the expression of DNA damage response and stem cell signaling proteins, and results in re-sensitization of chemoresistant OC cell lines to carboplatin.

Background

Fig 1: Possible mechanisms by which RAD6-mediated ubiquitin signaling regulates acquired chemoresistance and stemness contributing to OC recurrence.



Significance of the Study

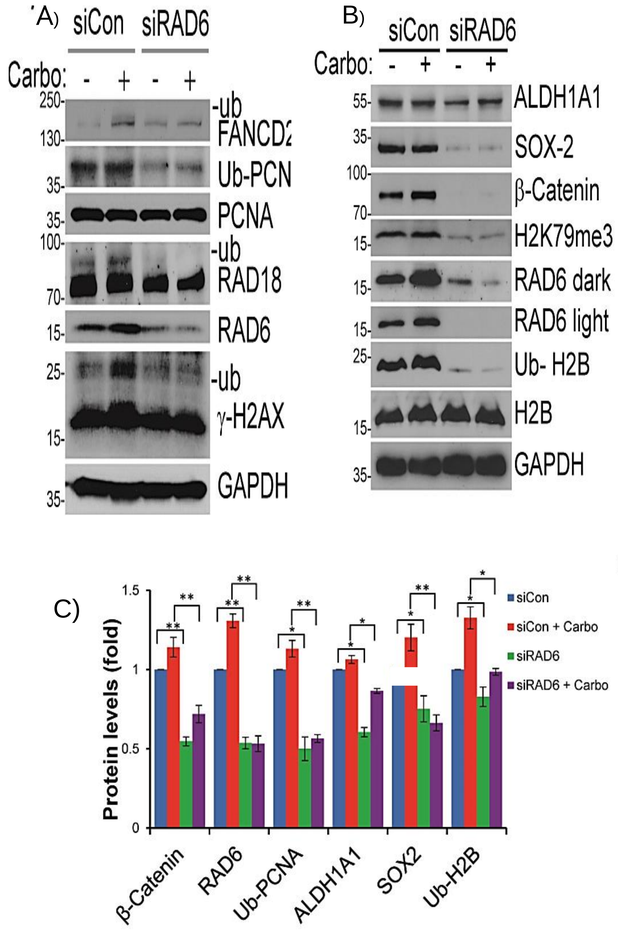
There is a need for a new, more efficacious drug that works in synergy with platinum based chemotherapeutic drugs . This would improve disease free survival rate in acquired chemoresistance in ovarian cancer.

Hypothesis

1. RAD6 can be a important therapeutic target to prevent and treat acquired chemoresistance.
2. A RAD6 inhibitor, TZ9 can increase efficacy of available platinum based chemotherapeutic drug.

Results

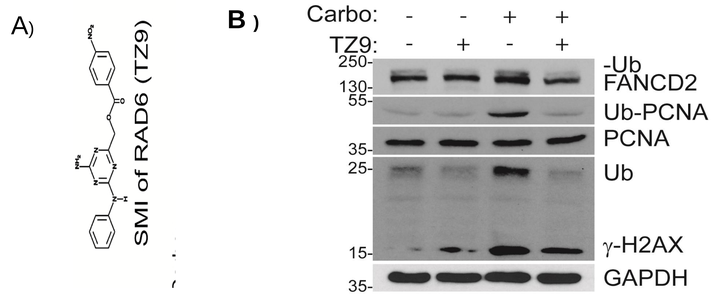
Figure 2: RAD6 mediated expression of ALDH1A1 & SOX2 after chemotherapy and RAD6 is a poor prognostic marker in OC.



A) RAD6 knockdown impairs carboplatin induced DDR in OV90 cells.
B) Histogram shows fold difference of protein expression affected by siRAD6.
C) RAD6 knockdown in OV90 cells decreases the expression of CSC signaling proteins.
E) Multiple siRNAs targeting either RAD6A or RAD6B were used to ensure the impact was not siRNA specific.

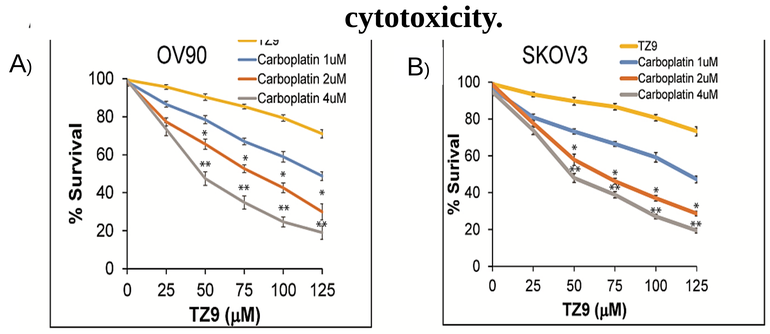
Results

Figure 3 : SMI of RAD6 (TZ9) induces DDR



A) Structure of the RAD6 SMI TZ9.
B) TZ9 blocks carboplatin-induced monoubiquitination of FANCD2, PCNA, and γH2AX.

Figure 4 : RAD6i TZ9 synergizes carboplatin induced cytotoxicity.



A) OV90 cells showed significant sensitization to carboplatin treatment at multiple concentrations of TZ9.
B) SKOV3 cells showed significant sensitization to carboplatin treatment at multiple concentrations of TZ9.

Conclusions

- Rad6 level increases in response to chemotherapy.
- Inhibition of RAD6 attenuates HR-mediated DSB repair and PCNA mediated TLS pathway.
- RAD6 SMI TZ9 attenuates RAD6 mediated FANCD2 monoubiquitination.
- TZ9 sensitizes OC cells to carboplatin.
- Together, these results suggest that RAD6 attenuates DNA repair in OC and potential therapeutic target to prevent acquired chemoresistance in OC.

Future Directions

Future Goal of our lab is to identify and develop a novel secondary molecular inhibitor for RAD6 with better efficacy in human for treatment of acquired chemoresistant in ovarian cancer .

Acknowledgements

1. Texas Tech University Health Sciences Center.
2. Somasagara RR, Spencer SM, Tripathi K, et al. RAD6 promotes DNA repair and stem cell signaling in ovarian cancer and is a promising therapeutic target to prevent and treat acquired chemoresistance. *Oncogene*. 2017;36(48):6680–6690. doi:10.1038/onc.2017.279