**RAD50 loss of function variants in the Zinc hook domain associated with higher risk of familial esophageal squamous cell carcinoma**

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**Objectives**

The genetic risk of rare deleterious variants with large effect associated with familial esophageal squamous cell carcinoma (ESCC) is unknown. Unbiased whole-exome sequencing (WES) analysis in 186 patients from endemic high-risk Henan in Northern China was utilized to prioritize RAD50 as one of the top candidates. RAD50 is a core component of the MRN1-RAD50-NBS1 (MRN) complex playing a pivotal role in coordinating DNA double-strand break (DSB) repair pathways. We aim to elucidate the dominant-negative impacts of two RAD50 mutants, located at the zinc hook and C-ATCase domains, on the ATM signaling axis and the possibility of harnessing these defects for a synthetic lethal approach involving ATM/ATR duo-disruption.

**Methods**

**Familial ESCC risk**

Next generation sequencing (NGS) analysis by WES and target capture validation of RAD50 was performed with a total of 1039 Henan individuals, 1044 familial ESCC, 1074 sporadic ESCC and 1171 controls. **p**-value was calculated by Fisher exact test, 1-sided.

**Functional characterization of RAD50 germline variants**

Lentivirus-mediated expression system was used to express wild-type (RAD50WT) and mutants (RAD50Δ227 and RAD50Δ124Δ77) in vitro for functional assays including cellular sensitivities towards CHK1 inhibitor AZD7762, replication stress, and DSB repair efficiency.

**Results**

**Fig. 3:** Lollipop schematic diagram of RAD50 LOF mutation distribution in (a) familial ESCC cases, (b) sporadic ESCC cases, and (c) controls from Henan.

**Fig. 4:** Sanger sequencing of RAD50 LOF germline mutations in five familial ESCC patients.

**Fig. 5:** Dominant negative effect of DSB repair by over-expression of Zinc hook RAD50Δ227 mutant.

**Fig. 6:** Dominant negative effect of over-expression of RAD50Δ227 and RAD50Δ124Δ77 in U2OS (a) sensitized cell viability (b) inhibited colony forming ability (c) increased pan-nuclear γH2AX response after AZD7762 (CHK1 inhibitor) treatment.

**Conclusions**

1. Two pathogenic RAD50 LOF variants, p.Q672X and the other recurrent p.K722fs variant at the zinc hook domain were significantly associated with increased risk of familial ESCC compared to sporadic ESCC and controls in Chinese populations.
2. The over-expression of RAD50Δ227 mutant contributes a dominant negative effect in DNA repair and sensitizes cell after treatment with CHK1 inhibitor, AZD7762.
3. Screening for the two pathogenic LOF RAD50 variants may have potential clinical utility to improve earlier cancer detection and prognosis among familial ESCC patients.

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**References**