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 MRE11-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-ATPcase domains, on the ATM signaling axis and the possibility of harnessing these defects for a synthetic lethal approach involving ATM/ATR duo-disruption.

Fig. 1: Schematic diagram showing the positions of mutations concerned, relative to key motifs of *RAD50*.

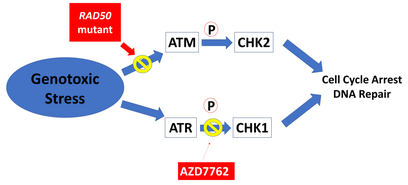


Fig. 2: Schematic diagram showing possible synthetic lethality along the ATM/ATR axes caused by *RAD50* mutant and *inhibition* of the ATR axis.[1]

Methodology

Famiial ESCC risk:

NGS analysis by WES and target capture validation of *RAD50* was performed with a total of 3103 Henan individuals, 1044 familial ESCC, 1074 sporadic ESCC and 1171 controls.[2] OR was calculated by Fisher exact test, 2 sided.
p <0.05 was considered statistically significant.

Functional characterization of *RAD50* germline variants:

Lentivirus-mediated expression system was used to express wild-type (*RAD50*^{WT}) and mutants (*RAD50*^{672X}, and *RAD50*^{L1264F}) *in vitro* for functional assays including cellular sensitivity towards CHK1/2 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.

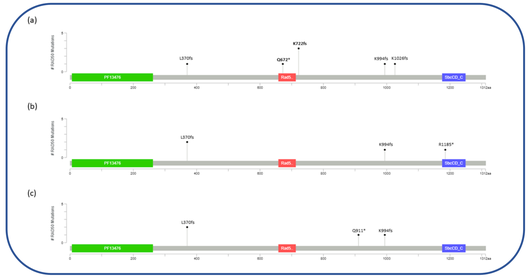


Fig. 4: Sanger sequencing of *RAD50* LOF germline mutations in five familial ESCC patients.

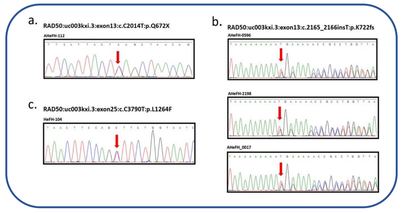
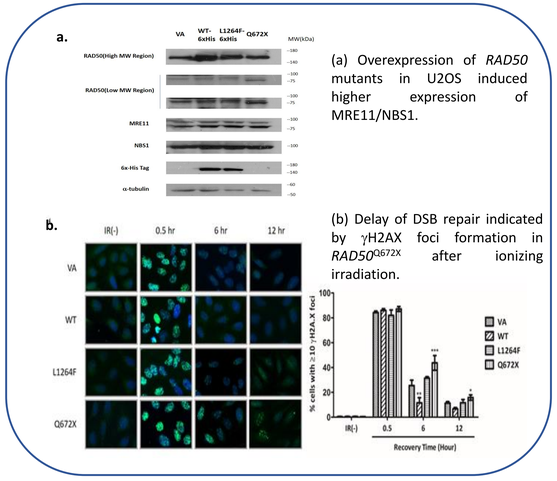


Fig. 5: Dominant negative effect of DSB repair by over-expression of Zinc hook *RAD50*^{Q672X} mutant.



(a) Overexpression of *RAD50* mutants in U2OS induced higher expression of MRE11/NBS1.

(b) Delay of DSB repair indicated by γH2AX foci formation in *RAD50*^{Q672X} after ionizing irradiation.

Fig. 6: Dominant negative effect of over-expression of *RAD50*^{Q672X} and *RAD50*^{L1264F} in U2OS (a) sensitized cell viability (b) inhibited colony forming ability; (c) increased pan-nuclear γH2A.X response after AZD7762 (CHK1 inhibitor) treatment.

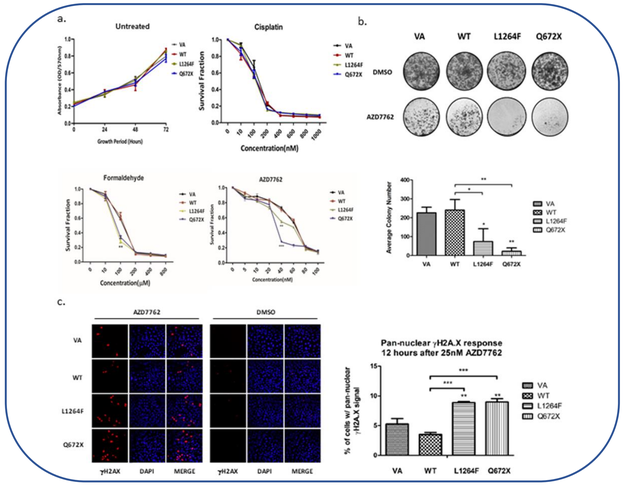


Table 2: Familial ESCC has significantly higher frequency of p.Q672X/p.K722fs mutations compared to sporadic ESCC and controls, East Asian and all populations from genomAD.

Mutation	FH+ ESCC (n=2,088)	Sporadic ESCC (n=2,148)	Controls (n=2,342)	p^a	OR[a]	gnomAD[d] East Asian (n=19,954)	p^b	OR[b]	gnomAD[d] All (n=282,670)	p^c	OR[c]
p.Q672X	0.045% (1)	0%	0%	0.32	inf	0%	0.095	Inf	0.00035% (1)	0.015	135.44
p.K722fs	0.14% (3)	0%	0%	0.032	inf	0.02% (4)	0.022	7.18	0.0014% (4)	$1.3x10^{-5}$	101.73
p.Q672X/ p.K722fs	0.19% (4)	0%	0%	0.010	inf	0.02% (4)	$4.1x10^{-3}$	9.57	0.0018% (5)	$3.5x10^{-7}$	108.51
All *RAD50* LOF	0.34% (7)	0.19% (4)	0.17% (4)	0.33	1.88	0.15% (30)	0.092	2.23	0.17% (427)	0.062	2.22

[a]p value and OR of Fisher Exact test comparing frequency of *RAD50* Zinc hook mutations from Henan Chinese familial ESCC with that from sporadic ESCC and controls (0/4490). [b]p value and OR of Fisher Exact test comparing frequency of *RAD50* Zinc hook mutations from Henan Chinese familial ESCC with that from East Asian population from genomAD. [c]p value and OR of Fisher Exact test comparing frequency of *RAD50* Zinc hook mutations from Henan Chinese familial ESCC with that from all populations from genomAD. [d]Frequencies of *RAD50* variants are exported from gnomAD, https://gnomad.broadinstitute.org/ on June 5, 2020.

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 *RAD50*^{Q672X} 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.

References

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2. Ko, J. M. et al. BRCA2 loss-of-function germline mutations are associated with esophageal squamous cell carcinoma risk in Chinese. Int J Cancer 146, 1042-1051, doi:10.1002/ijc.32619 (2020).

Acknowledgements

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