News Release

Title

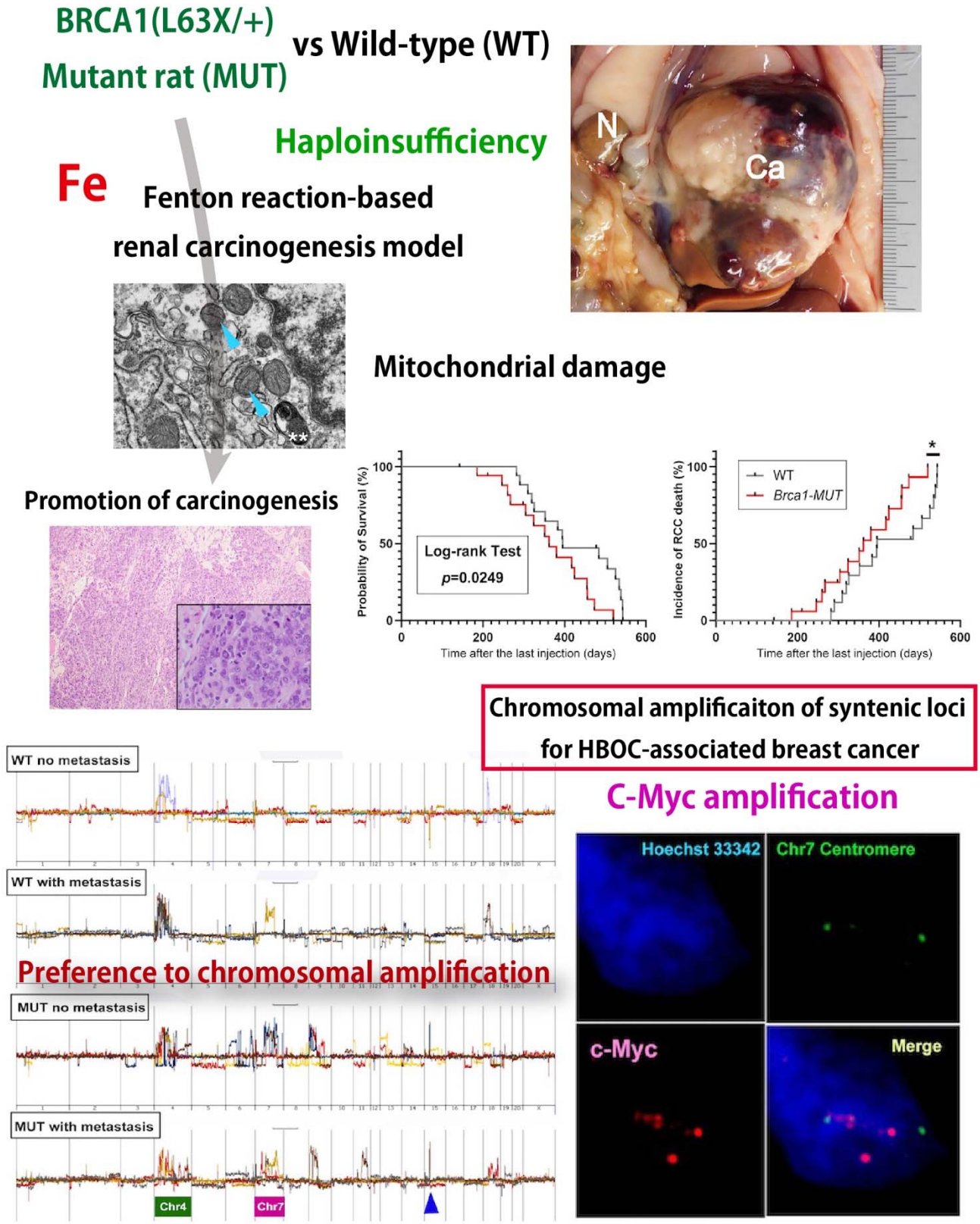
BRCA1 haploinsufficiency promotes chromosomal amplification under Fenton reaction-based carcinogenesis through ferroptosis-resistance

Key Points

- Rat BRCA1 haploinsufficiency promoted Fenton reaction-based renal carcinogenesis
- BRCA1 haploinsufficiency allowed chromosomal amplification under excess iron
- BRCA1 haploinsufficiency caused more mitochondrial damage with ferroptosis resistance

Summary

Germline-mutation in *BRCA1* tumor suppressor gene is an established risk for carcinogenesis not only in females but also in males. Deficiency in the repair of DNA double-strand breaks is hypothesized as a responsible mechanism for carcinogenesis. However, supporting data is insufficient both in the mutation spectra of cancers in the patients with *BRCA1* germline-mutation and in murine knockout/knock-in models of *Brca1* haploinsufficiency. Furthermore, information on the driving force toward carcinogenesis in *BRCA1* mutation carriers is lacking. Here we applied Fenton reaction-based renal carcinogenesis to a rat heterozygously knockout model of *BRCA1* haploinsufficiency (mutant [MUT] model; L63X/+). Rat MUT model revealed significant promotion of renal cell carcinoma (RCC) induced by ferric nitrilotriacetate (Fe-NTA). Array-based comparative genome hybridization of the RCCs identified significant increase in chromosomal amplification, syntenically similar to those in breast cancers of *BRCA1* mutation carriers, including c-*Myc*, in comparison to those in the *wild-type*. Subacute-phase analysis of the kidney after repeated Fe-NTA treatment in the MUT model revealed dysregulated iron metabolism with mitochondrial malfunction assessed by expression microarray and electron microscopy, leading to renal tubular proliferation with iron overload. In conclusion, we for the first time demonstrate that biallelic wild-type *BRCA1* provides more robust protection for mitochondrial metabolism under iron-catalyzed oxidative stress, preventing the emergence of neoplastic cells with chromosomal amplification. Our results suggest that oxidative stress via excess iron is a major driving force for carcinogenesis in *BRCA1* haploinsufficiency, which can be a target for cancer prevention and therapeutics.



Ca, renal cell carcinoma; N, non-tumorous kidney

Research Background

Historically, tumor suppressor genes were identified one by one through the genetic analysis of cancer-prone kins. *BRCA1*, cloned in 1994 as one of them, imposes a high risk, if one of the germline alleles is inactivated, not only for breast/ovarian carcinoma in females but also for breast, prostate and probably pancreatic/stomach cancer in males. Currently, this risk is clinically well recognized, and guidelines recommend risk-reducing bilateral mastectomy and salpingo-oophorectomy in the early life of women, which requires further consideration of novel strategies for higher quality of life.

Many mouse *Brca1* haploinsufficiency models have been generated since the 1990's. However, expected phenotypes were not obtained presumably due to the species difference and the short lifetime period. Recently, a rat heterozygous knockout model of *BRCA1* haploinsufficiency (mutant [MUT] model; *L63X*/+) has been established, which revealed a significant enhancement of radiation-induced mammary carcinogenesis in females (*T Imaoka, unpublished data*).

Excess iron is a risk for carcinogenesis. The association of breast cancer and excess iron has been previously suggested but is still controversial with deficiency of data. We thus far demonstrated that Fenton reaction-based repeated oxidative stress via ferric nitrilotriacetate (Fe-NTA) in rats causes renal cell carcinoma (RCC) with genetic alterations similar to those in humans. In the present study, we applied Fe-NTA-induced rat renal carcinogenesis to the rat *Brca1* MUT model to evaluate the involvement of BRCA1 haploinsufficiency in Fenton reaction-based carcinogenesis from the viewpoint of iron metabolism and the induced genetic alterations at the chromosomal level.

Research Results

1) Brca1 haploinsufficiency significantly promotes Fe-NTA-induced renal carcinogenesis

There was a significant promotional effect of renal carcinogenesis in *Brca1* haploinsufficiency in comparison to Wild-type (WT), which was regarded mainly to work at the promotional stage of carcinogenesis (median survival; WT 395 days *vs* MUT 362 days after the last injection; $P<0.05$). RCCs of MUT rats showed a higher but statistically not significant incidence of peritoneal invasion/dissemination (35.3% and 52.9%, $P=0.300$) with significantly higher Ki-67 cellular proliferation index.

2) Brca1 haploinsufficiency significantly increases chromosomal amplifications in Fe-NTA-induced RCCs

In WT RCCs with/without pulmonary metastasis, deletions were significantly prevalent in general but only centromeric portion of chromosome 4 showed significantly frequent amplification, where *c-Met* oncogene was located as we previously described. In contrast, MUT RCCs with/without pulmonary metastasis revealed significantly higher frequency of chromosomal amplification ($P<0.05$) in comparison to those in WT

3) Specific amplification of c-Myc oncogene in the RCCs of Brca1 MUT rats

We found *c-Myc* amplification in 4 cases (50.0%) of the eight MUT RCCs examined by aCGH. Only one case with pulmonary metastasis (12.5%) out of 8 WT RCCs showed *c-Myc* amplification. Further

FISH analysis on *c-Myc* locus confirmed significantly higher incidence of *c-Myc* amplification in the other MUT RCCs in comparison to WT RCCs (4/5 in MUT RCCs *vs* 0/5 in WT RCCs; in total, 8/13 [61.5%] in MUT RCCs *vs* 1/13 [7.7%] in WT RCCs; $P<0.01$) and revealed that *c-Myc* amplification included those in the extrachromosomal DNA (micronuclei).

4) Brca1 haploinsufficiency causes iron dysregulation in association with mitochondrial malfunction in the subacute phase of Fe-NTA-induced renal carcinogenesis

We performed expression microarray analysis at the subacute phase of carcinogenesis at 3 weeks (GEO accession: GSE198507). Differential analysis between WT and MUT at 3 weeks suggested the pathways involved in heme/hemoglobin, oxygen and iron, indicating that mitochondria are the major target organella. We further performed electron microscopic analysis on mitochondrial morphology, which disclosed that mitochondria are smaller with deformity whereas lysosomal fraction significantly increased even in the untreated MUT kidney. Significant increase in Tf production in the MUT kidney at 3 weeks of carcinogenesis protocol suggests an establishment of a regulatory system to avoid excess iron.

5) Brca1 haploinsufficiency generates carcinogenic environments with ferroptosis-resistance under iron-catalyzed persistent oxidative stress

8-Hydroxy-2′-deoxyguanosine (8-OHdG) was significantly increased at 3 weeks of carcinogenesis protocol in the MUT kidney with significantly higher Ki-67 cellular proliferation index in comparison to the WT kidney. HNEJ-1 antibody has been recently established as a tool to detect ferroptosis. Whereas the immunostaining was not different between WT and MUT in the untreated control kidney, WT showed significantly more intense immunostaining than MUT at 3 weeks of carcinogenesis protocol, suggesting that MUT kidney is more resistant to ferroptosis under the Fe-NTA-induced renal carcinogenesis protocol.

Future Perspective

BRCA1 in two normal alleles hence prevents chromosomal amplification under iron-catalyzed persistent oxidative stress, indicating that half an amount of BRCA1 is not sufficient to maintain the genome information unaltered under Fe(II)-catalyzed severe oxidative stress. Therefore, manipulating the iron metabolism, especially at the target organs, can be a preventive strategy of various carcinogenesis for the BRCA1 germline-mutated patients. Iron reduction as a measure either by iron chelating agent or phlebotomy was successful for the prevention of malignant mesothelioma at least preclinically. Of course, iron deficiency anemia due to menstruation or pregnancy has to be carefully ruled out for considering the procedures. Brca1(L63X/+) haploinsufficient rat provides us with a more plausible model than murine models to evaluate possible strategies to increase the quality of life of BRCA1 germline-mutated patients.

Publication

Yingyi Kong, Shinya Akatsuka, Yashiro Motooka, Hao Zheng, Zhen Cheng, Yukihiro Shiraki, Tomoji Mashimo, Tatsuhiko Imaoka and Shinya Toyokuni. BRCA1 haploinsufficiency promotes

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