A and B domains. Specifically, the integrity of a functional Walker B domain of RAD51D was reported to be essential for RAD51D–XRCC2 and RAD51C complex formation (37). We uncover a deleterious effect of c.620C>T;p.S207L on RAD51D HR activity where the key event is disruption of the interaction between RAD51D and XRCC2 paralogs of RAD51, both essential for RAD51 recruitment to DSBs upon DNA damage. This mechanism combined with the drastic effect that PARPi exhibited in the viability of c.620C>T;p.S207L-RAD51D cells further supports the notion that PARPi-targeted therapy will likely be effective in c.620C>T;p.S207L carriers. In addition, acquired resistance to PARPi in TNBC has recently been associated with RAD51 proficiency (44). Furthermore, the current study highlights the central importance of the RAD51D–XRCC2 interaction as a driver mechanism for tumorigenesis and consequently the pathogenicity likelihood of other deleterious missense mutations mapping within the Walker B domain of RAD51D.

The role of *RAD51D* mutations in breast cancer risk remains unresolved (3, 4, 45, 46), and a possible involvement of *RAD51D* specifically in TNBC seems plausible (47). Our results do not support an association between c.620C>T;p.S207L and breast cancer in FCs although our study has limited power to rule out such an association. The p.E233G (rs28363284) variant was previously studied in the context of breast cancer–associated risk, then functionally, but its association with breast cancer risk remains controversial (48), and its high frequency in public databases suggests it is a benign variant. In 2009, two studies from the same group described increased resistance by p.E233G to DNA-damaging agents upon p53 abrogation (49). However, our results show that p.E233G can restore DNA repair ability in RAD51D-depleted cells where p53 is present, suggesting therefore that p.E233G does not diminish RAD51D HR activity by itself.

The implications of this single mutation contributing to almost 4% of all ovarian HGSC in a founder population are significant and justify the need to establish surveillance and management programs for individuals at risk. Nowadays, with personalized health care as a global aim, there is open debate regarding the suitability of genetic testing at the population level, with different models under consideration. Population-specific approaches have their supporters (50), and this study certainly suggests that widespread testing of the FC population of Quebec for this and other founder variants could be an efficient, cost-effective approach to lessen the burden of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: B. Rivera, R. Siebert, P.N. Tonin, A. Orthwein, W.D. Foulkes

Development of methodology: B. Rivera, M. Di Iorio, S.L. Arcand, P.N. Tonin, A. Orthwein

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Rivera, M. Di Iorio, J. Frankum, S.L. Arcand, D. Grapton, E. Tomiak, V. Hastings, N. Hamel, R. Wagener, F.F. Hamdan, A. Dionne-Laporte, G. Zogopoulos, F. Rousseau, D. Provencher, G.A. Rouleau, J.L. Michaud, A.-M. Mes-Masson, S. Bens, S.A. Narod, M.R. Akbari, C.J. Lord, P.N. Tonin, A. Orthwein, W.D. Foulkes

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Rivera, M. Di Iorio, J. Frankum, J. Nadaf, S. Fahiminiya, S.L. Arcand, D.L. Burk, N. Hamel, R. Wagener, A.M. Berghuis, J.L. Michaud, J. Majewski, S. Bens, R. Siebert, C.J. Lord, P.N. Tonin, A. Orthwein, W.D. Foulkes

Writing, review, and/or revision of the manuscript: B. Rivera, M. Di Iorio, J. Nadaf, S. Fahiminiya, E. Tomiak, N. Hamel, R. Wagener, F.F. Hamdan, A. Dionne-Laporte, G. Zogopoulos, F. Rousseau, A.M. Berghuis, G.A. Rouleau, A.-M. Mes-Masson, S. Bens, R. Siebert, M.R. Akbari, P.N. Tonin, A. Orthwein, W.D. Foulkes

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B. Rivera, R. Wagener, O. Aleynikova, S. Bens, P.N. Tonin, W.D. Foulkes

Study supervision: R. Siebert, P.N. Tonin, W.D. Foulkes

Other (identified and prepared colorectal cases and extracted DNA from FFPE samples): S. Giroux

Acknowledgments

We thank D. Durocher for plasmids/cell lines, A.C. Gingras for pDEST constructs, J. Stark for U2OS DR-GFP cells, R. Greenberg for U2OS LacO cells, and G. Dellaire for CRISPR/Cas9 LMNA plasmids. We thank Jian Zhang for her initial work on this project, Laura Palma CGC for clinical care of the index family, Nelly Sabbaghian, Eric Bareke, Lilian Amrein, Manon de Ladurantaye, Lise Portelance, Pablo Gonzalez-Ginestet, and Alyssa Smith and the technicians of the molecular cytogenetic laboratory in Ulm, Germany, for technical support.

Grant Support

This work was financed in part by research grants to W.D. Foulkes from the Quebec Breast Cancer Foundation, Susan G. Komen, and the Canadian Institutes of Health Research (CIHR; FDN-148390) and a CIHR Project grant to A. Orthwein (361708). This work was supported in part by grants to P.N. Tonin from the Cancer Research Society of Canada and Fonds de recherche du Québec-Santé (FRQS). Tumor banking was supported by the Banque de tissus et données of the Réseau de recherche sur le cancer of the Fond de Recherche du Québec - Santé (FRQS), associated with the Canadian Tumor Repository Network (CTRNet). A. Orthwein also receives a Transition Grant from the Cole Foundation and is supported by the Sir Mortimer B. Davis Foundation from the Jewish General Hospital. B. Rivera was a fellow of Fundacion Martin Escudero international postdoctoral fellowship program and FQRNT- Quebec Merit Scholarship. M.R. Di Iorio was recipient of an MICRTP studentship program and the Dr. Clarke K. McLeod Memorial Scholarship. M.R. Akbari was supported by Canadian Cancer Society Research Institute. C.J. Lord receives funding from Cancer Research UK. F. Rousseau holds a salary award from the FRQ-Santé in evidence-based laboratory medicine. The Research Institute of the McGill University Health Centre and the Centre de recherche du Centre Hospitalier de l'Université de Montréal receive grant support from the FRQS.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 24, 2017; revised March 23, 2017; accepted June 6, 2017; published OnlineFirst June 23, 2017.

References

1. Constantinou P, Tischkowitz M. Genetics of gynaecological cancers. Best Pract Res Clin Obstet Gynaecol 2017 Jan 24. [Epub ahead of print].
2. Nielsen FC, van Overeem Hansen T, Sorensen CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer 2016;16:599–612.
3. Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet 2011;43:879–82.
4. Osher DJ, De Leeneer K, Michils G, Hamel N, Tomiak E, Poppe B, et al. Mutation analysis of RAD51D in non-BRCA1/2 ovarian and breast cancer families. Br J Cancer 2012;106:1460–3.
5. Wickramanayake A, Bernier G, Pennil C, Casadei S, Agnew KJ, Stray SM, et al. Loss of function germline mutations in RAD51D in women with ovarian carcinoma. Gynecol Oncol 2012;127:552–5.
6. Song H, Dicks E, Ramus SJ, Tyrer JP, Intermaggio MP, Hayward J, et al. Contribution of germline mutations in the RAD51B, RAD51C, and

RAD51D genes to ovarian cancer in the population. J Clin Oncol 2015;33:2901–7.
7. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461:747–53.
8. Spurdle AB, Healey S, Devereau A, Hogervorst FB, Monteiro AN, Nathanson KL, et al. ENIGMA–evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. Hum Mutat 2012;33:2–7.
9. Lindor NM, Guidugli L, Wang X, Vallee MP, Monteiro AN, Tavtigian S, et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). Hum Mutat 2012;33:8–21.
10. Couch FJ, Rasmussen LJ, Hofstra R, Monteiro AN, Greenblatt MS, de Wind N, et al. Assessment of functional effects of unclassified genetic variants. Hum Mutat 2008;29:1314–26.
11. Millot GA, Carvalho MA, Caputo SM, Vreeswijk MP, Brown MA, Webb M, et al. A guide for functional analysis of BRCA1 variants of uncertain significance. Hum Mutat 2012;33:1526–37.
12. Shimelis H, Mesman RL, Von Nicolai C, Ehlen A, Guidugli L, Martin C, et al. BRCA2 hypomorphic missense variants confer moderate risks of breast cancer. Cancer Res 2017;77:2789–99.
13. Ghadirian P, Robidoux A, Zhang P, Royer R, Akbari M, Zhang S, et al. The contribution of founder mutations to early-onset breast cancer in French-Canadian women. Clin Genet 2009;76:421–6.
14. Cybulski C, Carrot-Zhang J, Kluzniak W, Rivera B, Kashyap A, Wokolorczyk D, et al. Germline RECQL mutations are associated with breast cancer susceptibility. Nat Genet 2015;47:643–6.
15. Belanger MH, Dolman L, Arcand SL, Shen Z, Chong G, Mes-Masson AM, et al. A targeted analysis identifies a high frequency of BRCA1 and BRCA2 mutation carriers in women with ovarian cancer from a founder population. J Ovarian Res 2015;8:1.
16. Kotar KV, Osczevski K, Yamani L, Galvez M, Hamel N, Ferrara E, et al. One of six founder mutations account for nearly 75% of all BRCA1/2 mutations in French Canadian women attending two McGill University high-risk clinics. In: Proceedings of the 2005 American Society of Human Genetics Annual Meeting; 2005 Oct 25–29; Salt Lake City, UT. Bethesda, MD: ASHG; 2005.
17. Liu G, Ghadirian P, Vesprini D, Hamel N, Paradis AJ, Lal G, et al. Polymorphisms in GSTM1, GSTT1 and CYP1A1 and risk of pancreatic adenocarcinoma. Br J Cancer 2000;82:1646–9.
18. Castellsague E, Liu J, Volenik A, Giroux S, Gagne R, Maranda B, et al. Characterization of a novel founder MSH6 mutation causing Lynch syndrome in the French Canadian population. Clin Genet 2015;87:536–42.
19. Ancot F, Arcand SL, Mes-Masson AM, Provencher DM, Tonin PN. Double PALB2 and BRCA1/BRCA2 mutation carriers are rare in breast cancer and breast-ovarian cancer syndrome families from the French Canadian founder population. Oncol Lett 2015;9:2787–90.
20. Rudkin TM, Hamel N, Galvez M, Hogervorst F, Gille JJ, Moller P, et al. The frequent BRCA1 mutation 1135insA has multiple origins: a haplotype study in different populations. BMC Med Genet 2006;7:15.
21. Fahiminiya S, Witkowski L, Nadaf J, Carrot-Zhang J, Goudie C, Hasselblatt M, et al. Molecular analyses reveal close similarities between small cell carcinoma of the ovary, hypercalcemic type and atypical teratoid/rhabdoid tumor. Oncotarget 2016;7:1732–40.
22. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–303.
23. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
24. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol 2011;29:24–6.
25. Chae YK, Anker JF, Carneiro BA, Chandra S, Kaplan J, Kalyan A, et al. Genomic landscape of DNA repair genes in cancer. Oncotarget 2016;7:23312–21.
26. Nadaf J, Majewski J, Fahiminiya S. ExomeAI: detection of recurrent allelic imbalance in tumors using whole-exome sequencing data. Bioinformatics 2015;31:429–31.
27. Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY, Stratton MR. A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun 2015;6:8683.
28. Wojnarowicz PM, Oros KK, Quinn MC, Arcand SL, Gambaro K, Madore J, et al. The genomic landscape of TP53 and p53 annotated high grade ovarian serous carcinomas from a defined founder population associated with patient outcome. PLoS One 2012;7:e45484.
29. Ventura RA, Martin-Subero JI, Jones M, McParland J, Gesk S, Mason DY, et al. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn 2006;8:141–51.
30. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10:845–58.
31. Schrodinger LLC. The PyMOL Molecular Graphics System, Version 1.8. 2015.
32. Hinz JM, Tebbs RS, Wilson PF, Nham PB, Salazar EP, Nagasawa H, et al. Repression of mutagenesis by Rad51D-mediated homologous recombination. Nucleic Acids Res 2006;34:1358–68.
33. Pinder J, Salsman J, Dellaire G. Nuclear domain `knock-in' screen for the evaluation and identification of small molecule enhancers of CRISPR-based genome editing. Nucleic Acids Res 2015;43:9379–92.
34. Orthwein A, Noordermeer SM, Wilson MD, Landry S, Enchev RI, Sherker A, et al. A mechanism for the suppression of homologous recombination in G1 cells. Nature 2015;528:422–6.
35. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285–91.
36. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
37. Wiese C, Hinz JM, Tebbs RS, Nham PB, Urbin SS, Collins DW, et al. Disparate requirements for the Walker A and B ATPase motifs of human RAD51D in homologous recombination. Nucleic Acids Res 2006;34:2833–43.
38. Murai J, Huang SY, Renaud A, Zhang Y, Ji J, Takeda S, et al. Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Mol Cancer Ther 2014;13:433–43.
39. Loveday C, Turnbull C, Ruark E, Xicola RM, Ramsay E, Hughes D, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet 2012;44:475–6.
40. Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet 2010;42:410–4.
41. Janavicius R. Founder BRCA1/2 mutations in the Europe: implications for hereditary breast-ovarian cancer prevention and control. EPMA J 2010;1:397–412.
42. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016;534:47–54.
43. Suwaki N, Klare K, Tarsounas M. RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. Semin Cell Dev Biol 2011;22:898–905.
44. Liu Y, Burness ML, Martin-Trevino R, Guy J, Bai S, Harouaka R, et al. RAD51 mediates resistance of cancer stem cells to PARP inhibition in triple-negative breast cancer. Clin Cancer Res 2017;23:514–22.
45. Baker JL, Schwab RB, Wallace AM, Madlensky L. Breast cancer in a RAD51D mutation carrier: case report and review of the literature. Clin Breast Cancer 2015;15:e71–5.
46. Gutierrez-Enriquez S, Bonache S, de Garibay GR, Osorio A, Santamarina M, Ramon y Cajal T, et al. About 1% of the breast and ovarian Spanish families testing negative for BRCA1 and BRCA2 are carriers of RAD51D pathogenic variants. Int J Cancer 2014;134:2088–97.
47. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large

Published OnlineFirst June 23, 2017; DOI: 10.1158/0008-5472.CAN-17-0190

triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol 2015;33:304–11.
48. Dowty JG, Lose F, Jenkins MA, Chang JH, Chen X, Beesley J, et al. The RAD51D E233G variant and breast cancer risk: population-based and clinic-based family studies of Australian women. Breast Cancer Res Treat 2008;112:35–9.
49. Nadkarni A, Rajesh P, Ruch RJ, Pittman DL. Cisplatin resistance conferred by the RAD51D (E233G) genetic variant is dependent upon p53 status in human breast carcinoma cell lines. Mol Carcinog 2009;48:586–91.
50. Foulkes WD, Knoppers BM, Turnbull C. Population genetic testing for cancer susceptibility: founder mutations to genomes. Nat Rev Clin Oncol 2016;13:41–54.

Published OnlineFirst June 23, 2017; DOI: 10.1158/0008-5472.CAN-17-0190

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Functionally Null *RAD51D* Missense Mutation Associates Strongly with Ovarian Carcinoma

Barbara Rivera, Massimo Di Iorio, Jessica Frankum, et al.

Cancer Res 2017;77:4517-4529. Published OnlineFirst June 23, 2017.