Review of: "The tumour microenvironment in BRCA1/BRCA2 hereditary breast cancer and the role of epigenetics in its regulation"

Janice Pluth[1]

1 University of Nevada, Las Vegas

Potential competing interests: No potential competing interests to declare.

This manuscript is a review article that states its focus is to: *1) explore the differences in tumour microenvironment between hereditary and sporadic breast cancer and 2) uncover the epigenetic mechanisms that also perpetuate such tumour microenvironments and hence contribute to the tumourigenesis of hereditary breast cancer, such as the role of LSD-1 in the suppression of BRCA1 gene expression."*

I appreciate the author has attempted cover a large area in this review article and provided some interesting and insightful information. I would say however, some key information is lacking, if the objectives are what is stated. For example, in many sections it is not noted how sporadic and BRCA1/2 defective tissues are different in various factors relating to tumor microenvironment. If it is not known than this should then be stated. In addition, the review also does not focus on all epigenetic mechanisms that relate to changes in the microenvironment, but rather seems to mainly focus on LSD-1.

A side note, the manuscript also suffers from issues with sentence structure which sometimes change the meaning of what is being said. I would suggest having a native English speaker go through the entire manuscript and ensure proper structure to relay the correct meaning. A few instances are described but not all were cataloged.

Abstract

1. In the first sentence it is noted that having BRCA1/2 variants "greatly increases the risk of being diagnosed with breast cancer." I believe what the author means is that having a defective BRCA1/2 gene increases the risk of breast cancer.
2. The final sentence of abstract does not appear correct as written *'While LSD1 has no direct role in mutations of BRCA1 or BRCA2 genes, its epigenetic influence shines light on the role of LSD1 inhibitors as a potential mode of therapy in the management of breast cancer, particularly for BRCA1/2 PV carriers."*

LSD-1 is a gene that when upregulated is associated with poor prognosis in some breast cancer patients, but it would not be correct to state that its role in epigenetics "sheds light" on the role of LSD1 inhibitors in treating breast cancer in BRCA1/2 carriers. I think what is meant is that this gene maybe prognostic in breast cancer, and due to its general role in epigenetics, may indicate that its inhibition would be beneficial in treating breast cancer.

Introduction

1. First sentence "*Hereditary breast cancer refers to a pluralistic group of genes that, when inherited, greatly increase the risks of breast cancer.*"

"Hereditary breast cancer" is not a "group of genes", again this seems to be a grammatical issue with sentence structure.

In addition, the word "pluralistic" is not typically used in this context. It is more often used in terms of society and refers to people within that society and their thoughts or beliefs.

1. "*In women who have been diagnosed with breast cancer, the risk of the contralateral breast developing cancer is also significantly higher.*"

Given the author was discussing specifically BRCA1/2 carriers in this paragraph, this last sentence is unclear, is this specifically indicating carriers, or all breast cancer patients. This should be clarified.

1. *"While the genetic basis of BRCA1/2 hereditary breast cancer is well-studied, the role of epigenetic mediators in the tumorigenesis of these hereditary breast cancers is also worth exploring because the expression and suppression of these gene mutations do have a component of epigenetic regulation [4]."*

Sentence structure/ wording issue, "gene mutations do not have a component of…"

1. *"A key player in such epigenetic dysregulation is Lysine-specific demethylase 1 because of its high levels of expression in hormone-negative breast cancer"*

The high level of expression ALONE is not why it is a key player in epigenetic dysregulation, it is due to what it does, not just it's expression level, this should be more clearly stated.

Section 2.1

1. I believe it would be correct to state "prostate cancer", not "prostatic cancer".

Section 2.4

1. *"BRCA1 PVs have been shown to alter the TME by directly enhancing epithelial-to-mesenchymal transition (EMT) in tumour cells."* This statement requires a reference.
2. Linderman and Visvader, in Breast Cancer Res. 2011; 13(2): 306, discuss the reason for slug overexpression in BRCA1 mutant cell lines and it should be more fully discussed if it is mentioned.

Section 2.5

1. It is mentioned that cells containing BRCA PV that CAFs are differentially regulated, however it is unclear what system this was studied in, was it a study in humans, in mice, in cells? How was this defined, should also be described. More should be expounded on how this may differ with sporadic breast cancer as that was an aim of this paper. The author moves back and forth between BRCA PV tissue results and sporadic breast cancer and do not clearly state where this the evidence for each statement is coming from specific to BRCA PV or sporadic tumors and the studies supporting

each. Thus, it would be better if the author specifically stated how activation or action of CAF are different for those individuals with BRCA PV and those that develop breast cancer sporadically.

Section 2.6

1. *"*Breast cancer cells normally stimulate surrounding adipose stromal cells to produce aromatase, an enzyme that catalyses the formation of oestrogen, by producing factors like IL-6 and Prostaglandin E2. This paracrine loop is kept in control by *BRCA1,* which inhibits aromatase gene expression in the stromal cells.

As this is written, italicizing BRCA1 indicates that the gene is inhibiting aromatase, which would not be correct, as assuming it is the protein product that would cause inhibition. In general, genes names are italicized while protein names are not. For example, INS refers to the human insulin protein while *INS* refers to the human insulin gene. However, throughout the manuscript it seems BRCA1/2 are always italicized regardless of if the author is referring to protein or gene.

1. The author notes "Although tumours carrying BRCA1 pathogenic variants usually do not express oestrogen receptor alpha, it has been shown that these cells can still respond to the increased oestrogen independent of oestrogen receptor expression [35]."

It would be good if the author included discussion regarding the importance of estrogen alpha receptor and why it is thought that even without its expression estrogen is overexpressed in BRCA1 defective cells.

2.7 Angiogenesis

1. Starting a new paragraph with a transition word like "additionally" is not thought to be grammatically correct.
2. Just as was done with Hif1, the author should also describe VEGF function first before then defining how it is deferentially regulated in BRCA1 patients' cells then readers have this background.
3. There was no mention of rationale for HIF overexpression in tissues from individuals with BRCA1/2 PV, only VEGF, so it seems it would be important to discuss also why this is seen for HIF given the section heading.
4. This sentence is not clear *"C-terminal binding protein-interacting protein (CtIP) and Zinc finger and BRCA1-interacting protein with KRAB domain-1 (ZBRK1)" is it the Zinc finger domain in BRCA1 that binds to CtIP"*. It would be good to be clearer regarding which domains, on which protein, are interacting with each other and how.
5. It would be good to provide some background regarding focal adhesion is and what that has to do with angiogenesis.

2.8 Immune cells

1. This section starts by by noting tissues with BRCA1/2 PV have negatively impacted immune systems. But then the author moves to describing BRCA1/2 role in DNA DSB and genomic instability. The DNA repair role would seem to better fit under another heading prior to this, where there is a description of the normal role of these proteins in a cell.
2. In reading this section it seems the author is implying micronuclei are the source of all inflammation and genomic instability. However, micronuclei are not the only source of inflammatory signaling and genomic instability. This should be clarified. In addition, micronuclei do not typically lead to mutations, more typically this is due to breakage of

chromosome and miss-repair leading to aberrations. Additionally, asymmetric chromosome aberrations often result in fragments and loss of DNA, but these cells typically die. It is also not clear how cells with micronuclei, if unable to support DNA replication can then lead to additional DNA damage. Typically, micronuclei result in apoptotic cell death. These points should all be clarified in this section.

3. It would be good to better describe how DNA:RNA hybrids and genome-embedded ribonucleotides are formed, and their relevance to this section, which is stated to be “immune cells”.
4. What “enhanced immune response” is should be described and noted how it is defined, measured and quantified in reference to STING activation.
5. Cytoplasmic DNA is not just caused by micronuclei rupture. Also, it is not clear how a polymerase would affect micronuclei formation. This should be better described.

Section 5.1

1. *“The role of LSD-1 is evident in the global H3K9me2 reduction seen in the EMT process*” It is important to explain and correctly state that LSD1 is involved in epigenetic regulation and may play a role in EMT-related epigenetic changes, but it is not the sole or main reason for these changes, as EMT is a complex process with contributions from various epigenetic factors. EMT is a highly orchestrated and multifaceted process that involves numerous epigenetic modifications, including changes in DNA methylation patterns, histone modifications, and the expression of various epigenetic regulatory proteins.

Section 7

1. *“The differences in methylation status also affect the responsiveness of these cancers to immunotherapy [5]. Specifically, poly (ADP-ribose) polymerase (PARP) inhibitors such as olaparib have been shown to be an effective adjunct therapy as part of the OlympiA trial to improve survival outcomes in BRCA1/2 hereditary breast cancers [118] [119].”*

It is unclear why after differences in methylation status do the author then discusses PARP inhibitors? The general understanding is that in cells with defective BRCA genes, that are unable to perform homologous recombination to repair DNA, inhibition of PARP can be lethal, as they become heavily reliant on PARP-mediated repair mechanisms for survival. So how does this relate to the methylation status? This needs to be clearer as well as the mechanism of PARP inhibitors action in these cells.

I hope these comments are helpful in editing this manuscript. Due to time constraints, I was unable to supply comments for all sections, but would again point to general issues in the beginning in editing sections not directly mentioned.