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

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Abstract

Hereditary genetic conditions such as the autosomal-dominant Hereditary Breast and Ovarian Cancer (HBOC) syndrome, in which genes such as *BRCA1* and *BRCA2* pathogenic variants (PVs) are inherited, greatly increase the risks of being diagnosed with breast cancer. Breast cancers in *BRCA1/2* PV carriers tend to be more aggressive and have poorer prognoses in part because these PVs influence the tumour microenvironment and facilitate tumourigenesis through their interactions with stromal cells and immune cells, promoting epithelial-mesenchymal transition and angiogenesis, and influencing oestrogen levels. In addition, *BRCA1* PVs also contribute to breast cancer by exerting epigenetic effects on cells, such as DNA methylation and histone acetylation, thereafter suppressing the expression of proto-oncogenes and promoting cytokine dysregulation. Amongst epigenetic regulators, lysine-specific demethylase 1 (LSD-1) has been touted to be a master epigenetic regulator of both transcription repression and activation, regulating both *BRCA1* and, to a lesser extent, *BRCA2* genes epigenetically. Upregulation of LSD-1 in cancer patients has generally been associated with a poorer prognosis, and LSD-1 contributes to the development of breast cancer in *BRCA1/2* PV patients through a plethora of mechanisms, including the perpetuation of a hypoxic environment and through direct suppression of *BRCA1* gene expression. 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.

Keywords: hereditary breast cancer, *BRCA1* and *BRCA2* pathogenic variants, tumour microenvironment, epigenetics, lysine-specific demethylase 1, breast cancer management.

1. Introduction

Hereditary breast cancer refers to a pluralistic group of genes that, when inherited, greatly increase the risks of breast cancer. The most common genes implicated in hereditary breast cancer are the *BRCA1* and *BRCA2* genes. It is estimated that about 10% of breast cancers result from inherited mutations in the *BRCA1* and *BRCA2* genes [1]. Locally in Singapore, female *BRCA 1/2* PV carriers are at a 45-70% risk of developing breast cancer by the age of 70[2], compared

to just 6% in the general female population [3]. In women who have been diagnosed with breast cancer, the risk of the contralateral breast developing cancer is also significantly higher.

While the genetic basis of *BRCA1/2* hereditary breast cancer is well-studied, the role of epigenetic mediators in the tumourigenesis 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]. Epigenetics refers to the study of changes in gene function that do not involve a change in DNA sequence or chromosomal aberrations. These modifications may even be inherited mitotically or meiotically [5]. A key player in such epigenetic dysregulation is Lysine-specific demethylase 1 because of its high levels of expression in hormone-negative breast cancer [6].

This two-part review aims 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.

2. Hereditary breast cancer

2.1. Hereditary Breast and Ovarian Cancer (HBOC) syndrome

Hereditary Breast and Ovarian Cancer (HBOC) syndrome is an autosomal dominant genetic condition which predisposes affected women to developing breast and ovarian cancer, men to prostatic cancer, and both to other cancers like pancreatic cancer and melanoma due to the inheritance of certain pathogenetically variant genes, most commonly *BRCA1* and *BRCA2* [7].

2.2. BRCA1 and BRCA2 pathogenic variants

BRCA1 and *BRCA2* are tumour suppressor genes responsible for repairing double-strand DNA breaks via the homologous recombination repair (HRR) pathway. Research done over the past decades has identified nearly 70,000 human BRCA variants, of which only a small proportion are pathogenic [8]. These pathogenic variants are expressed in an autosomal dominant pattern with incomplete penetrance. Population-based studies put its penetrance for breast cancer at 45% to 65% [9][10].

2.3. The impact of BRCA1/2 PVs on the tumour microenvironment

BRCA1/2 PVs have been postulated to have effects on the tumour microenvironment (TME) of resulting hereditary breast cancers, such that the tumour microenvironment of these cancers differs from that of breast cancers that develop sporadically. These differences could be a contributory factor for the increased likelihood of the development of breast cancers seen in HBOC syndrome, as well as their increased aggressiveness and worse prognosis compared to sporadic breast cancers.

The TME is largely determined by the mutations of the tumour cell and the effects of infiltrating inflammatory cells including lymphocytes, plasma cells, dendritic cells, macrophages and neutrophils within the tumour core [11][12], while at the tumour periphery, stromal fibroblasts, myoepithelial cells, adipocytes and endothelial and vascular/lymphatic endothelial cells are the main contributors to the TME [13][14].

Metastasis of tumour cells results in new tumour microenvironments at these sites of metastasis[15][16].

Various soluble factors, such as cytokines, hormones, growth factors and enzymes, and physical factors, such as pH and oxygen content, also play a role in tumour progression in the breast and at distant sites [11][14].

2.4. Epithelial to mesenchymal transition

BRCA1 PVs have been shown to alter the TME by directly enhancing epithelial-to-mesenchymal transition (EMT) in tumour cells.

Physiologically, the EMT process plays a crucial role in embryogenesis and wound healing. During this, epithelial cells lose their polarity and intercellular adhesions but acquire proteins found in mesenchymal cells, which facilitates travel to other sites.

However, EMT is implicated pathogenically in tumourigenesis as well, and the loss of E-cadherin is a key step in this process. One factor that can repress the transcription of E-cadherin and thus promote cancer is the TWIST protein. It has been shown that *BRCA1* binds to the TWIST promoter, suppressing its activity and inhibiting the epithelial-to-mesenchymal transition process [17]. Thus, *BRCA1* PVs result in TWIST overexpression and tumourigenesis.

Slug is another such factor that can repress the transcription of E-cadherin, and it has been reported to be upregulated in the presence of *BRCA1* PVs despite *BRCA1* not being a transcriptional repressor of it[18][19].

BRCA1 PVs have also been thought to induce aberrant interaction of breast cancer cells with other cell surface and cytoskeletal proteins responsible for the regulation of EMT, such as P-cadherin, beta-catenin, vimentin and cytokeratins [20].

Ultimately, this means that *BRCA1* PVs can cause EMT in luminal stem cells and induce their dedifferentiation, not only promoting the expansion of basal and cancer stem cells but also increasing the risk of formation of basal-like tumours [21][22][23][24]. Basal-like tumours have the worst prognosis as it is the most aggressive and metastatic out of all breast cancer subtypes [25][26].

2.5. Stromal cells

BRCA1 PVs also influence the tumour microenvironment through its effects on surrounding stromal cells.

In sporadically occurring breast cancer, mesenchymal stromal cells have been shown to promote EMT[27][28]. These

mesenchymal stromal cells are upregulated by *BRCA1* PVs, thereby further increasing the metastatic potential of tumours carrying *BRCA1* PVs [29][30][31].

Apart from mesenchymal stromal cells, *BRCA1* PVs can also influence cancer-associated fibroblasts (CAFs) to further promote metastasis of cancer cells. CAFs are fibroblasts with increased proliferation and the ability to synthesise dysfunctional tumour suppressor proteins [32]. In sporadic breast cancers, they normally promote tumour progression by enhancing angiogenesis, growth and invasion of the tumour, through the secretion of enzymes altering the extracellular matrix, such as vascular endothelial growth factors and matrix metalloproteinases [33].

However, in the presence of *BRCA1* PVs, these CAFs become activated and transform into metastasis-associated fibroblasts (MAFs), which can increase their expression of EMT markers such as Ezrin and CCL5, to further induce metastatic changes in breast cancer cells and augment tumour proliferation, migration, and invasion [34].

2.6. Oestrogen

Furthermore, local oestrogen levels in the tumour microenvironment of breast cancer cells containing *BRCA1* pathogenic variants are elevated, thus increasing oestrogen-dependent tumour proliferation and growth.

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. Thus, with *BRCA1* PVs, there is oestrogen overproduction [30]. 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].

2.7. Angiogenesis

Additionally, *BRCA1/2* PVs can enhance tumour angiogenesis. Angiogenesis is necessary for the continued growth of tumours and is regulated by pro-angiogenic and anti-angiogenic factors. As cancer cells proliferate, their metabolic demand increases, and they require a greater oxygen supply. This causes relative oxygen shortages within rapidly growing tumours, resulting in the formation of a hypoxic environment, causing increased activity of hypoxic inducible factors (HIF).

Under normoxic conditions, the alpha subunit of HIF1, HIF1α, is hydroxylated, which causes it to be recognised and degraded by proteasomes [36]. As oxygen is required for this hydroxylation, hypoxia stabilises HIF1α. One of the HIF target genes transcribes vascular endothelial growth factor (VEGF), which is a key driver of angiogenesis.

It has been shown that VEGF and HIF are more highly expressed in *BRCA1/2* PVs than sporadic breast cancers [37], and this has been attributed to the fact that *BRCA1* can bind to the VEGF gene promoter and suppress its activity, inhibiting its transcription [38].

Apart from VEGF, it is postulated that *BRCA1* can affect other pro-angiogenic factors, particularly angiopoietin 1, by forming a repressive complex with C-terminal binding protein-interacting protein (CtIP) and Zinc finger and *BRCA1*-interacting protein with KRAB domain-1 (ZBRK1) which then inhibits the expression of angiopoietin 1 [39].

Other studies have also demonstrated that *BRCA1/2* PVs downregulate miRNAs with possible onco-suppressive properties, specifically miR-573 and miR-578, which regulate the signalling pathways of VEGF, HIF and Focal Adhesion [40].

2.8. Immune cells

Arguably, the most important effect *BRCA1/2* PVs have on the tumour microenvironment is its influence on immune cells.

BRCA1/2 are key for maintaining genomic integrity through homologous recombination to repair double-stranded DNA breaks. BRCA1 initiates the process of homologous recombination by controlling the DNA-end resection at these DNA breaks, whereas *BRCA2* functions downstream of it by loading the RAD51 recombinase to facilitate the actual recombination process [41].

In many cancer cells exhibiting genomic instability, such as those with*BRCA1/2* PV, there is increased inflammatory signalling in the tumour microenvironment. This is due to the formation of micronuclei from damaged DNA undergoing mitosis [42][43], which are mis-segregated chromosomes surrounded by a single lipid bilayer, not part of the main nucleus. These chromosomal fragments are acentric and thus are unable to support faithful DNA replication and DNA repair, leading to additional DNA damage, mutations and even chromothripsis within the micronuclei [44][45][46]. These micronuclei persist in daughter cells and may eventually be reincorporated into the main nucleus after several divisions, resulting in chromoanagenesis [47].

Alternatively, but more commonly, these micronuclei rupture due to the improper organisation of their nuclear lamina, releasing the micronucleus DNA into the cytoplasm [48]. The cell responds to such ‘self’ DNA that ends up in the cytoplasm similarly to how it would to microbial DNA, as such DNA is also recognised by cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), which functions as part of the cell’s innate immune response [49][50][51]. Once it recognises micronucleus DNA in the cytosol, cGAS is activated and catalyses the production of cyclic 2'3' GAMP, which triggers STING-dependent inflammatory signalling, resulting in the release of IFN-1 [52].

The accumulation of cytoplasmic nucleic acids and activation of the cGAS/STING pathway is prevented by TREX1 nuclease, which degrades cytoplasmic DNA [53]. Additionally, DNA:RNA hybrids can be processed by RNase H1/H2, while genome-embedded ribonucleotides can be hydrolysed by RNAseH2 [50][54][55][56]. However, once this process is overwhelmed, cGAS is recruited and activated [57][58][59], and its activation is further enhanced should the micronuclei rupture [50].

STING activation leads to NF-kb signalling and the phosphorylation of Interferon regulatory factor 3 (IRF3), both of which ultimately lead to an enhanced immune response through the increased release of cytokines, especially interferons, inhibiting further tumour development.

However, cells with *BRCA1/2* PVs have a way to suppress cGAS signalling and evade immune clearance. One of the main ways it does this is by preventing the generation of cytoplasmic DNA. This is achieved by utilising alternative but non-conservative DNA repair pathways to repair double-strand breaks. Specifically, the Polymerase θ (POLQ)-mediated alternative end-joining and RAD52-mediated single-strand annealing are commonly utilised alternative repair pathways in cancer cells carrying *BRCA1/2* PVs [60]. It has been found that POLQ is upregulated in HR deficient cancers like in *BRCA1/2* PVs [61], and that inhibiting POLQ results in micronuclei formation and IFN signalling[62]. Cip2A and TopBp1 are also implicated in *BRCA1/2* PV cells, which form a complex with Mdc1 to tether chromosome fragments during mitosis, preventing the generation of micronuclei [63][64].

Another possible way *BRCA1/2* PV cells clear cytoplasmic DNA is through the utilisation of RNaseH1 and TREX1 enzymes, as mentioned previously. However, their significance as a compensatory response is unclear as of now [53][65].

Alternatively, the immune response is blunted through the modulation of the tumour microenvironment. Various immunosuppressive cytokines have been found to be upregulated in BRCA1/2 PV cells, such as IL-10, which prevents the maturation of dendritic cells [66] as well as CCL-9 and IL-23, due to the increased expression of*C-MYC* [67], which also suppresses IFN signalling and thus the activation of pro-inflammatory cytokines [68][69][70].

Interestingly, the activation of the cGAS/STING pathway in*BRCA1/2* PV cells has been shown to upregulate ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) [71], which regulates cGAS signalling. ENPP1 mainly functions to break down extracellular adenosine triphosphate (ATP), resulting in the formation of AMP [72], which is then broken down further by 5'-Nucleotidase Ecto (NT5E) to form adenosine [71], which is immunosuppressive within the TME. This is believed to play a role in the increased infiltration of regulatory T cells within the TME seen in BRCA1/2 PV cancers [73][74].

3. Epigenetic modification mechanisms in hereditary breast cancer

While *BRCA1/2* PVs have an extensive influence on tumourigenesis by affecting the tumour microenvironment, the differences in epigenetics of hereditary and sporadic breast cancers also predispose hereditary *BRCA1* PV carriers and, to a lesser extent, *BRCA2* ones to the development of cancer. They do so by contributing to and perpetuating the tumour microenvironment of hereditary breast cancer. Generally, three classes of epigenetic regulation exist to regulate gene expression [5][75][76].

3.1. DNA methylation

DNA methylation takes place primarily in cytosine nucleotide bases within CpG dinucleotide sequences, which are more often found in the promoter regions of silenced genes than ones with active transcription [77][78]. Methylated cytosine can then block the binding of transcription factors and recruit other repressors of transcription, including histone deacetylases that lead to chromatin remodelling. However, DNA methylation has also been found to take place across non-CpG

sequences [5]. The mechanism of DNA demethylation, on the other hand, is less understood than that of methylation, although it has been implicated in a variety of conditions, such as cardiovascular diseases and malignancies [75].

In BRCA1 PV breast cancers, the promotor region of the oestrogen receptor alpha (ER) is highly methylated compared to the sporadic breast cancers [79][80]. Such a phenomenon provides a possible explanation for the low expression of ER in hereditary breast cancers. In both normal breast tissue and in breast cancer, methylation levels of the promoter regions of tumour suppressor genes such as *BRCA1*, *BRCA2* and *ESR1* were higher in both *BRCA1/2* PV carriers than non-carriers [79][81][82].

Conversely, in *BRCA1* PV breast cancer, various studies have shown that there is a global DNA hypomethylation in tumour cells with respect to sporadic breast cancers [80][83][84]. The hypomethylation of proto-oncogenes allows for an increase in the expression of these genes, hence facilitating tumourigenesis. Examples of proto-oncogenes that have been shown to be upregulated include *RAD9*, a gene implicated in cell cycle control and DNA repair, as well as c- which codes for transcription factors in cell signalling [84].

3.2. Histone modification

Histones can undergo a plethora of post-translational epigenetic modifications such as acetylation or deacetylation, methylation or demethylation, and phosphorylation or dephosphorylation that consequently alter gene expression. The best-studied histone modifications are the acetylation and methylation of lysine residues on histones H3 and H4 histones. Notably, unlike histone acetylation and deacetylation, which promote and repress transcription, respectively, the effects of histone methylation and demethylation are more ambiguous, depending on the position of the lysine residue and extent of methylation [5][75][77][78].

In most sporadic breast cancers with intact *BRCA1* and *BRCA2* genes, the C-terminal domain of the BRCA protein interacts with histone deacetylases to promote the deacetylation of histones as well as other genes [81][83]. For instance, HDAC1 complexes with *BRCA1* to deacetylate genes are involved in the non-homologous recombination pathway of DNA repair, while HDAC2 complexes with *BRCA1* to deacetylate histones H2A and H3 [85]. In hereditary *BRCA1/2* PV breast cancers, the knockout of BRCA proteins results in impaired histone deacetylation [86]. An example of the impact of a lack of deacetylation of histones H2A and H3 would be the upregulation of the miR-155 promoter and the overexpression of micro-RNA 155 (miR-155), which leads a dysregulation in cytokine signalling pathways as well as the facilitation of epithelial-mesenchymal transition [86].

3.3. Regulatory non-coding RNA action

Non-coding RNAs such as small inhibitory RNAs (siRNAs) and microRNAs (miRNAs) repress transcription by promoting DNA methylation and histone modifications mediated by proteins such as Argonaute. The mechanisms behind which these regulatory non-coding RNAs regulate gene expression remain to be elucidated [5][87][88][89][90].

Studies have shown that in *BRCA1/2* PV hereditary breast cancer, certain miRNAs, such as miR-148 and miR-335, were

downregulated, while certain ones were upregulated, such as miR-21 and miR-206. While such a correlation is weak [79] and the significance of this remains unclear, given the role of mi-RNAs in repressing translation[91], such a difference in epigenetic regulation could have implications in the tumourigenesis of hereditary breast cancer as compared to sporadic breast cancers [92].

4. Epigenetic maintenance of a conducive tumour microenvironment in hereditary breast cancer

Noteworthy enzymes involved in the perpetration of a conducive tumour microenvironment by means of epigenetic regulation in hereditary breast cancer include histone methyltransferases such as the enhancer of zeste homologue 2 (EZH2) that represses target gene expression [4] and lysine methyltransferase 2 (KMT2) that promotes the expression of oestrogen-dependent oncogenes like the epidermal growth factor [93] as well as histone methylases such as the JumonjiC family [4], of which the enzyme LSD-1 features prominently, and will be the main focus of the review.

4.1. Lysine-specific demethylase 1

LSD-1 is a prototypical histone demethylase enzyme involved in epigenetic processes that has been implicated in the pathogenesis of breast cancer as well as many other tumours. [5][75][77][78]. It has also been associated with a poor cancer prognosis [75][77][78][94][95][96][97]. In terms of enzymatic activity, LSD-1 catalyses the demethylation of mono-methylated or di-methylated lysine 4 on histone H3 histone (H3K4me1 and H3K4me2, respectively). However, depending on the substrate, LSD-1 has been shown to have epigenetic effects on both transcriptional activation [5][77][78] as well as repression [5][75][98][99][100].

Besides this, LSD-1 can also demethylate non-histone proteins, such as tumour suppressor proteins, to affect epigenetic influences. Examples include the demethylation of K370 lysine residue of *p53*, the demethylation of lysine 442 of *MYPT1*, which is an important regulator of the dephosphorylation of the retinoblastoma protein (*pRb1*), as well as the demethylation of lysine 185 of the *E2F1* transcription factor. These all work to suppress the expression and effects of the tumour suppressor proteins.

5. LSD-1 and the tumour microenvironment in breast cancer pathogenesis

Due to its versatility as an epigenetic modulator in both transcription repression and activation, LSD-1 has been touted to be a master regulator controlling cellular homeostasis [5]. As such, it has a complementary role alongside *BRCA1/2* PVs and is inextricably intertwined with cellular processes that contribute to tumorigenesis in breast cancer. A few such processes will be expounded upon below.

5.1. Epithelial-mesenchymal transition

The EMT has been elaborated on previously when discussing the tumour microenvironment of breast cancer. The role of LSD-1 is evident in the global H3K9me2 reduction seen in the EMT process. By binding to the SNAI-1 protein, which, together with the Slug protein, represses E-cadherin, LSD-1 contributes to the loss of cellular adhesions between cancer cells and augments their ability to invade and metastasise [78].

5.2. Downregulation of tumour suppressor proteins

Insofar as a few tumour suppressor proteins, including the*p53* and *E2F1*, are non-histone substrates of LSD-1, the demethylation of these proteins by excessive LSD-1 enzymes inadvertently leads to downregulation and, by extension, the promotion of tumorigenesis in breast cancer [101][102].

5.3. Regulation of hypoxia

A hypoxic tumour microenvironment promotes the growth of tumours and angiogenesis. Under hypoxic conditions, LSD-1 regulates hypoxia through the demethylation of hypoxia-inducible factor-1(HIF-1) to stabilise it [5]. This counteracts the action of SET-7/9, which catalyses the mono-methylation of HIF-1a and promotes its degradation. LSD-1 also indirectly contributes to the stability of HIF-a through a series of interactions with other proteins, such as the demethylation of the HIF-1a-interacting protein RACK-1, as well as the inhibition of HIF-1a hydroxylation, which mediates its degradation [5][103].

5.4. Metabolic reprogramming

Raised LSD-1 levels have been correlated with a shift from mitochondrial to glycolytic respiration, which is a hallmark of most cancer cells. Increased glycolysis and increased glucose uptake were also associated with HIF-1a levels, of which LSD-1 stabilises as an adaptation to the hypoxic microenvironment. This allows for tumour cells to proliferate without the consumption of oxygen during respiration [78]. Through the demethylation of genes such as acyl-CoA dehydrogenase medium chain (ACADM), LSD-1 represses mitochondrial respiration. Conversely, it has been shown that decreasing LSD-1 levels is associated with a decrease in glucose uptake and glycolysis, consequently activating mitochondrial respiration [78][104]

5.5. The role of LSD-1 in tumour suppression

Interestingly, LSD-1 has also been shown to contribute to tumour suppression by its association with the NuRD complex. As a subunit of the NuRD complex, LSD-1 inhibits TGF-signalling genes, thus inhibiting EMT. This, in turn, has implications for the suppression of cancer metastasis. Recent studies have found that LSD-1-NuRD complexes are associated with the suppression of luminal breast cancer metastasis [75].

While this presents an ostensible equivocality to the role of LSD-1 in tumourigenesis, it should be noted that the majority of its functions have been implicated in cancer pathogenesis. Raised LSD-1 levels remain a poor prognosticating factor for

many cancers, including breast cancer. The interplay between its roles in both promoting and inhibiting EMT and how these processes can be reconciled remains to be understood and uncovered [105].

6. LSD-1 and hereditary breast cancer

6.1 The association of LSD-1 with aggressive subtypes of breast cancer

Of the four molecular subtypes of breast cancer – basal-like, luminal A, luminal B and HER2 positive – the type most strongly associated with LSD-1 overexpression thus far has been basal-like breast cancers, which, as previously mentioned, are also more likely to occur in individuals carrying BRCA1 pathogenic variants. Basal-like breast cancers frequently do not express hormonal receptors and HER2, with many basal-like breast cancers being triple-negative breast cancers (TNBC) and vice versa [106]. These cancers have the worst prognosis, with many patients being of a younger age and having a larger tumour size on diagnosis [107].

Not only is the overexpression of LSD-1 linked to more aggressive subtypes of breast cancer, but it is also associated with poorer outcomes in these subtypes of breast cancer when compared to the same subtypes of breast cancer but with less LSD-1 expression. It has been demonstrated that a higher degree of LSD-1 overexpression in basal-like and HER2-positive breast cancers is correlated to shorter recurrence-free survival and higher hazard ratios for recurrence [108].

This is in stark contrast to sporadic breast cancers, in which the luminal A breast subtype, which has the best prognosis, occurs most commonly [108].

6.2. Downregulation of BRCA1 and BRCA2

The overexpression of LSD-1 in breast cancer has been correlated with a downregulation of *BRCA1*, especially in aggressive cancers such as basal-like and triple-negative breast cancer [5]. This is because, in these cancers, the Wnt signalling is upregulated, leading to an upregulation of the expression of the transcription repressor slug together with an accumulation of catenin. The SNAG domain on Slug interacts with LSD-1, forming a complex that binds to the promoter region of BRCA1 and represses its expression [109].

The effects of downregulating *BRCA1* by LSD-1 are arguably more pronounced in hereditary breast cancer with *BRCA1* PV because of the additional component of genetic instability, fewer functional BRCA proteins, and an increased likelihood of loss of heterozygosity, in which the wild-type alleles of the *BRCA* genes are lost. Moreover, *BRCA1/2* PV carriers are more likely to develop more aggressive cancers like triple-negative breast cancer, especially *BRCA1*-related tumours, which often have a similar profile to triple-negative breast cancer, such as the feature of marked overexpression of LSD-1 [109][110][111].

Moreover, LSD-1 also mediates hypoxia-induced H3K4 demethylation at *the BRCA1* gene promoter, leading to decreased *BRCA1* gene expression. By stabilising HIF-a and perpetuating the hypoxic microenvironment in breast cancer, LSD-1

also allows for E2F4/p130 complexes to repress the transcription of the *BRCA1* gene [112][113]. The hypoxic tumour microenvironment also contributes to the downregulation of BRCA2 [114]. Observational studies have shown that levels of expression of either *BRCA* gene were closely linked to the other and that women with *BRCA1/2* PV have similar or overlapping regulatory pathways [115]. By extrapolation, it can be hypothesised that by downregulating *BRCA1*, LSD-1 might also play a part in the downregulation of *BRCA2*, the mechanism of which remains to be investigated. Once again, the effects of downregulating functioning BRCA2 gene copies are more pronounced in patients with *BRCA2* PV.

7. Therapeutics in breast cancer

Hereditary *BRCA1/2* PV cancers are associated with a poorer prognosis compared with sporadic ones and a preponderance to the development of other cancers, such as cancer of the contralateral breast and ovarian cancer [2][116]. As such, the management of these cancers is differentiated from that of sporadic ones. For instance, prophylactic management and screening are important components of hereditary breast cancer management [2]. In terms of the management of cancer, there are higher rates of mastectomies as well as chemotherapy-only adjuvant and neoadjuvant regimes in *BRCA1/2* PV-related breast cancers than in sporadic breast cancers. On the other hand, the chances of hereditary breast cancer patients receiving hormone therapy without chemotherapy are lower [117]. This is owing to the fact hereditary breast cancers predispose patients to triple-negative and basal-like cancers. 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].

7.1. LSD-1 inhibitors in breast cancer therapy

Due to LSD-1 being implicated in several cancers, LSD-1 inhibitors, many of which are derived from monoamine oxidase (MAO) inhibitors owing to their structural similarity, have been developed as a therapeutic modality [75][77][78]. One of the first such inhibitors to be identified was tranylcypromine (TCP), an irreversible inhibitor of LSD-1. Others include the reversible inhibitors GSK354 and GSK2879552 [5]. Moreover, natural bioactive compounds such as flavones, xanthones and melatonin have all been found to have LSD-1-inhibiting properties and to offer promising results in the development of new LSD-1 inhibitors.

Chemical LSD1 inhibitors have been successfully used to block the growth of embryonic stem cells, pluripotent carcinomas like teratomas and embryonic carcinoma, as well as leukaemia [77]. In terms of breast cancer, the LSD-1 inhibitor INCB059872, together with immunotherapy such as anti-programmed cell death ligand 1 drug (anti-PD-L1), enhanced the efficacy of such immunotherapy agents and general anti-tumour efficacy [5]. Other studies have also found LSD-1 inhibition to increase the number of PD-L1 receptors on epithelial breast cancer cells and triple-negative breast cancer cells [120]. Given the lack of responsiveness of breast cancer to immunotherapy due to the absence of a high tumour mutational burden and lymphocytic infiltration [5], the addition of LSD-1 inhibitors to the armamentarium of anti-tumour drugs represents a promising new therapy [121].

7.2. The potential of LSD-1 inhibition as prophylactic therapy for hereditary breast cancers

For women who are carriers of *BRCA1/2* PV but are without breast cancer, offered risk management options for breast cancer comprise either intensified risk surveillance or risk-reducing measures, including risk-reducing bilateral mastectomy (RRBM) and chemoprevention [2]. Chemoprevention through medications is only given on a case-to-case basis and comprises selective oestrogen receptor modulators such as Tamoxifen.

The role of LSD-1 in breast cancer tumourigenesis needs no further reiteration, and in view of the current successes of LSD-1 inhibitors, the role of LSD-1 inhibition as an epigenetic intervention is a potential area of future research that remains to be uncovered.

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