Review

The Breast Cancer Protooncogenes HER2, BRCA1 and BRCA2 and Their Regulation by the iNOS/NOS2 Axis

Katie Lin 1, Stavroula Baritaki 2, Silvia Vivarelli 3,4, Luca Falzone 5, Aurora Scalisi 6, Massimo Libra 3,7 and Benjamin Bonavida 1,*

1 Jonsson Comprehensive Cancer Center, Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA; katielin@mednet.ucla.edu
2 Laboratory of Experimental Oncology, Division of Surgery, School of Medicine, University of Crete, 71003 Heraklion, Crete, Greece; baritaks@uoc.gr
3 Department of Biomedical and Biotechnological Sciences, University of Catania, 95030 Catania, Italy; silvia.vivarelli@unime.it (S.V.); m.libra@unict.it (M.L.)
4 Occupational Medicine Section, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, 98123 Messina, Italy
5 Epidemiology and Biostatistics Unit, IRCCS Istituto Nazionale Tumori “Fondazione G. Pascale”, 80131 Naples, Italy; l.falzone@istitutotumori.na.it
6 Italian League against Cancer, 95030 Catania, Italy; a.scalizi@unict.it
7 Research Centre for Prevention, Diagnosis and Treatment of Cancer, University of Catania, 95030 Catania, Italy
* Correspondence: bbonavida@mednet.ucla.edu

Abstract: The expression of inducible nitric oxide synthase (iNOS; NOS2) and derived NO in various cancers was reported to exert pro- and anti-tumorigenic effects depending on the levels of expression and the tumor types. In humans, the breast cancer level of iNOS was reported to be overexpressed, to exhibit pro-tumorigenic activities, and to be of prognostic significance. Likewise, the expression of the oncogenes HER2, BRCA1, and BRCA2 has been associated with malignancy. The interrelationship between the expression of these protooncogenes and oncogenes and the expression of iNOS is not clear. We have hypothesized that there exist cross-talk signaling pathways between the breast cancer protooncogenes, the iNOS axis, and iNOS-mediated NO mutations of these protooncogenes into oncogenes. We review the molecular regulation of the expression of the protooncogenes in breast cancer and their interrelationships with iNOS expression and activities. In addition, we discuss the roles of iNOS, HER2, BRCA1/2, and NO metabolism in the pathophysiology of cancer stem cells. Bioinformatic analyses have been performed and have found suggested molecular alterations responsible for breast cancer aggressiveness. These include the association of BRCA1/2 mutations and HER2 amplifications with the dysregulation of the NOS pathway. We propose that future studies should be undertaken to investigate the regulatory mechanisms underlying the expression of iNOS and various breast cancer oncogenes, with the aim of identifying new therapeutic targets for the treatment of breast cancers that are refractory to current treatments.

Keywords: breast cancer; iNOS/NOS2; nitric oxide; HER2; BRCA1; BRCA2; TNBC

1. Introduction

Several reports have described the role of inducible nitric oxide synthase (iNOS) in various cancers and its clinical significance [1–6]. Such reports have indicated that the expression of iNOS and nitric oxide (NO) in some cancers is anti-tumorigenic. For example, it was found that in mice, iNOS is anti-tumorigenic in colon cancer cells [7,8]. Additionally, when iNOS was transfected into murine melanoma cells, iNOS inhibited tumorigenesis and metastasis [9,10]. However, other reports demonstrated the pro-tumorigenic activity of NO. One study found that iNOS is induced both in the epithelial cells and the environmental...
stroma community of a tumor, which promotes tumorigenesis [3,11]. Findings in breast cancer also reported that iNOS expression was correlated with tumor progression and was of prognostic significance [12–16].

Various mechanisms have been postulated for the contrasting role of iNOS/NO in cancers. Specifically, it was found that the dual role of iNOS is dependent on its concentration, cell type, and environment [3]. High concentrations of iNOS-induced NO were found in human ovarian cancer cell lines [10,17]. It was also found that there were higher levels of iNOS expression in less differentiated breast carcinomas [10,18]. The underlying mechanisms of the relationship between high iNOS expression and the pathogenesis of human breast cancer are not well defined. This review aims to address these mechanisms. We hypothesize that there are some cross-talk signaling pathways between iNOS expression and the expression of breast cancer protooncogenes: HER2, BRCA1, and BRCA2. In this report, we describe the molecular regulations of the expression of the breast cancer protooncogenes HER2, BRCA1, and BRCA2 and the role of iNOS expression in those regulations. In addition, we performed bioinformatic analyses to delineate the interrelationships between iNOS and the expression of the oncogenes above.

2. Breast Cancer

Breast cancer is a heterogeneous disease that is the global leading cause of cancer-related death in women [19,20]. It is heterogeneous because it consists of many different subtypes that have different clinical outcomes [20–22]. As a result, there must be continuing research advancements in diagnosing and treating breast cancer [20–22]. Specifically, it is important to study tumor progression and resistance to treatments at the clinical and molecular levels [20–22]. Different subtypes of breast cancer include alterations in the gene expression of oncogenes such as HER2/neu, Ras, and PI3K [23].

Protooncogenes are the genes in normal cells that drive the cell cycle forward through cell proliferation and differentiation [24]. However, when protooncogenes undergo gain-of-function mutations, they become permanently activated, becoming oncogenes [24]. These oncogenes stimulate uncontrollable cell division, which promotes tumorigenesis in the development of cancer [23]. These protooncogenes that turn into oncogenes are HER2, BRCA1, and BRCA2.

2.1. Breast Cancer Protooncogenes

2.1.1. HER2

There are many oncogenes involved in the development and progression of breast cancer. HER2 is an oncogene that is located on chromosome 17q and encodes the 185 kDa tyrosine kinase receptor protein [23,25]. HER2 is a protein that is a member of the epidermal growth factor receptor (EGFR) family [26,27]. The overexpression of HER2 is found in breast, ovarian, lung, and oral cancers [27–34]. Specifically, overexpression of HER2 has been found in 20%–30% of invasive human breast cancer cases [23,28,35].

Transcriptional Regulation of HER2

It has been shown that HER2 is overexpressed through the transactivation of its promoter [36,37]. One study found that E1A is capable of repressing the HER2/neu gene at the transcriptional level. E1A represses HER2 by inhibiting transcription factors that bind to and activate the promoter region of HER2 [37,38]. Specifically, E1A binds to p300/CBP and inactivates the p300/CBP complex on HER2 [37,39]. Since p300/CBP is an enhancer-binding protein for HER2, the inhibition of p300/CBP represses HER2 gene expression [37,40,41].

Another study investigated the effects of the SV40 large T-antigen (c-myc) on HER2 gene expression [42,43]. Hung et al. characterized the rat, mouse, and human HER2 promoters and used transient transfection assays [41,43–47]. Hung et al. found that when c-myc was transfected into the HER2 promoter regions, the rat HER2 promoter was inhibited while the human HER2 gene expression was unaffected [42,43,48,49]. As a result, Hung
et al. concluded that there must be continuing large T gene therapy on HER2 in human cancer cells to study its regulation of the overexpression of HER2 [43].

Additionally, the effects of epigallocatechin-3 gallate (EGCG) on HER2/neu in breast cancer cells were investigated [50]. Pianetti et al. analyzed the focal mammary tumors of transgenic mice that overexpressed HER2/neu [50,51]. EGCG is a main antioxidant in green tea, and this study fed mice green tea to study the effects of EGCG on HER2/neu [50,52]. Through the use of the Akt kinase assay and immunoblot analysis, Pianetti et al. found that EGCG slows the proliferation and reduces the growth of tumor cells [50]. EGCG does this by reducing basal receptor tyrosine phosphorylation of HER2/neu [50]. EGCG also inhibits HER2/neu signaling pathways, including the pathway that activates NF-kB [50,53]. NF-kB causes inflammation in tumor cells, which contributes to the progression of cancer [50,53]. As a result, EGCG inhibits the growth and proliferation of mammary tumors by inhibiting HER2/neu signaling pathways [50]. It also methylates HER2/neu, which results in the inhibition of HER2/neu gene and protein expression [50].

Epigenetic Regulation of HER2

There are also epigenetic factors that affect the expression of HER2/neu. A study investigated how DNA methylation and demethylation affect HER2/neu expression in ovarian cancer [54,55]. The promoter region of HER2/neu has six CpG sites [55,56]. In this experiment, Hattori et al. methylated these CpG sites using specific primers in PCR that targeted positions 2, 206, 213, 299, and 513 in the HER2/neu promoter region [55,57]. Hattori et al. then used immunohistochemistry and found that methylating the HER2/neu promoter resulted in the silencing of the gene [55]. These results were then compared to the demethylated HER2/neu promoter, where it was found that demethylation increased HER2/neu gene expression [54,55]. This study was conducted by comparing the samples of 43 human ovarian cancers to 43 human non-cancerous ovarian tissues [55]. As a result, it was found that methylation of the promoter region of HER2/neu downregulates HER2 gene expression while demethylation upregulates HER2 gene expression [55].

Post-Transcriptional Regulation of HER2

There are also post-transcriptional factors that regulate HER2/neu gene expression [58]. Small interfering RNAs (siRNAs) are double-stranded RNAs that induce post-transcriptional silencing of specific targeted genes [58]. Yang et al. transfected 20bp miRNAs into HER2/neu of human breast cancer cells [58,59]. From the transfection, it was found that retrovirus-mediated RNA interference using siRNAs resulted in the gene silencing of HER2/neu [58]. As a result, siRNAs are able to decrease the expression of HER2/neu mRNA and protein, which can lead to inhibited tumor growth [58]. The siRNAs are synthesized to be homologous to regions of the HER2 exons [60]. Choudhury et al. also synthesized them to be homologous to other HER family members [60].

2.1.2. BRCA1

Wild-Type BRCA1

BRCA1 functions as a tumor suppressor gene that controls cell cycle checkpoints and repairs DNA within a normal cell [61]. However, in breast and ovarian cancer cells, BRCA1 is mutated [61]. Normally, BRCA1 represses estrogen receptor α (ER-α), which is a transcription factor that can mediate tumorigenesis upon exposure to estrogen [61–63]. BRCA1 is a gene that has many important cellular functions within the body, including DNA repair, cell cycle regulation, and transcriptional regulation of other genes [64–66]. Specifically, it binds to complex DNA structures and regulates the G2/M checkpoint protein Chk1 [67–69]. It also can induce large-scale chromatin unfolding, which contributes to transcription and repair within the cell [68,70].

When BRCA1 is mutated, it no longer represses ER-α, which results in the development and progression of triple-negative breast cancer [61–63,71].
Additionally, BRCA1 normally functions to control the migration of breast cancer cells, which limits metastasis within the body [61]. The mechanism behind this is that BRCA1 ubiquitinates ezrin, radixin, and moesin (ERM), which is a membrane protein complex that promotes metastasis [61,72]. However, when BRCA1 is mutated in breast cancer cells, it can no longer degrade the ERM complex [61,73]. This results in the overexpression of ERM proteins, which leads to the metastasis of breast cancer cells from the prime site to the secondary site [61].

It was also found that in mammary epithelial cells, mutated BRCA1 promotes cell motility and invasion [61,74]. In these cells, BRCA1 enhances the protein expression of transcription factors Snail 1 and Snail 2, known as slug [61,75,76]. The mechanism behind BRCA1’s regulation of Snail 1 and Snail 2 remains unknown [75]. Upregulation of Snail 1 and Snail 2 elevates the level of epithelial-to-mesenchymal phenotype (EMT) in the cells [61,76]. EMT changes the shape of cells to be more spindle-shaped, making them highly motile [61]. As a result, the mutation of BRCA1 results in EMT being induced, which promotes metastasis of breast cancer cells [61]. Another study found that BRCA1 has a role in maintaining genetic stability in a normal cell [77].

Specifically, there are three domains of the BRCA1 protein that are mutated in many cancer cases [78,79]. One such domain is the RING domain that encompasses amino acids 1-109 (exons 2-7), which functions as an E3 ubiquitin ligase [78,79]. To test this, one study mutated the BRCA1 RING domain by replacing it with alanine in genetically engineered mice [79–81]. Clark et al. found that the E3 ubiquitin ligase activity and therefore the RING domain plays a large role in tumor suppression [79–81].

Another domain is the region encoded by exons 11-13, which encompasses over 65% of the BRCA1 sequence. It also encodes the nuclear localization sequences (NLS) and has binding sites for proteins such as the retinoblastoma protein (RB), c-Myc, Rad50, and Rad51 [79,82]. Researchers are continuing to investigate the exact structure and function of this domain, but Clark et al. have a general idea from assessing the functions of its binding partners [79,82]. One of its binding proteins includes Myc, which is a transcription factor for a large number of genes, where binding to this domain would activate other genes [79,82]. Additionally, Rad50, Rad51, and PALB2 are involved in DNA repair. Lastly, RB controls cell cycle progression [79,82]. As a result, the researchers concluded that the domain of exons 11-13 plays a large role in the tumor suppressor function of BRCA1 [79,82].

Finally, there is the BRCT domain from exons 16-24 (amino acids 1650-1863), which is a phosphoprotein-binding domain [79,83]. It has specificity for proteins that are phosphorylated by ATM/ATR kinases, which are both activated by DNA damage [79,83]. The phosphoproteins that bind to the domain are BACH1, ChIP, and CCDC98/abraxas [79,84–86]. The main function of the BRCT domain is to recognize the sequence pSer-X-X-Phe in its phosphorylated binding partners in order to modulate interactions between BRCA1 and phosphoproteins [79,84–86]. BRCT phosphorylates target phosphoproteins in response to DNA damage in the cell [79,84–86]. As a result, the mutation of this domain can lead to a loss of tumor suppressor function in BRCA1 [79].

However, when there is a deficiency of BRCA1, there is uncontrolled cell division, increased proliferation, and tumorigenesis [71–73]. Estradiol (E2) is an abundant estrogen that is found in women, and it was shown to induce high levels of BRCA1 during puberty and pregnancy in mice [87–89]. This is because E2 stimulation leads to the estrogen receptor (ER-α) and p300 binding to an activator protein site on the BRCA1 promoter [89,90]. As a result, estrogen regulates BRCA1 activity. However, it was also found that BRCA1 transcriptionally regulates ER-α, creating a negative feedback loop [89,91]. BRCA1 inhibits transcriptional activation of ER-α by deregulating p300, which is a coactivator of ER-α [89,91]. However, ER-α signaling pathways promote the proliferation and differentiation of breast and ovarian tissues [89]. When BRCA1 is mutated, most of the time it results in the inability of BRCA1 to repress ER-α [89,91]. As a result, tumorigenesis occurs, resulting in BRCA1-related malignancies in breast, ovary, and prostate tissues [66,89].
Mutated BRCA1

BRCA1 and BRCA2 mutations are known to contribute to the susceptibility of breast and ovarian cancers. Through genetic linkage studies, BRCA1 has been localized to chromosome 17q [92,93]. Specifically, BRCA1 accounts for 81% of breast–ovarian cancer families, while BRCA2 only accounts for 14% of these families [93–95]. However, studies have shown that the combination of BRCA1 and BRCA2 mutations accounts for the most high-risk breast cancer families [93,96]. This was found by using genetic markers to test for BRCA1 and BRCA2 mutations in families from the Breast Cancer Linkage Consortium (BCLC) [93].

When BRCA1 is mutated, it was found that it can indirectly promote tumorigenesis by increasing the mutation rates of other genes [70,97]. Xu et al. found that BRCA1 can induce mutations in the Trp53 tumor suppressor gene [70,98,99]. Specifically, the mutated BRCA1 inhibits the expression of Trp53 in mammary gland tissues [70,98,99]. As a result, the inhibition of Trp53 results in increased cell proliferation and therefore tumor formation in mammary cells [70,98,99]. Yasmeen et al. analyzed the effects of the 3450delCAAG BRCA1 mutation on breast carcinogenesis and metastasis [67]. Specifically, it was found that in human mammary epithelial cell lines, this mutation in BRCA1 promoted cell cycle progression, cell motility, and cell invasion [67]. Yasmeen et al. tested this by comparing the mutated cell lines to the normal BRCA1 cell lines using transfection, Western blotting techniques, and RT-PCR [67].

It was found that mutated BRCA1 deregulates cell cycle progression at the G0/G1 phase [67,100,101]. This is because BRCA1 deregulates cyclins A, E, D1, D2, and D3 along with their catalytic partners Cdk2, Cdk4, and Cdk6 [67,100,101]. As a result, mammary epithelial cells surpass the G0/G1 cell cycle checkpoint, allowing for uncontrolled cell cycle progression [67,100,101]. Additionally, mutated BRCA1 deregulates the expression of cavelon-1, P-cadherin, E-cadherin, and Id-1, which are major regulators of cell invasion and metastasis [67]. As a result, the 3450delCAAG BRCA1 mutation deregulates regulators of cell cycle progression, cell motility, and cell invasion, which can contribute to the metastasis of breast and ovarian cancers [67].

Another study found that mutated BRCA1 affects the expression of the β-subunit of human chorionic gonadotropin (β-hCG) in breast cancer [102–104]. β-hCG has been shown to inhibit apoptosis, promote cell invasion and proliferation, and act as an immunosuppressant [61,105–107]. When BRCA1 is mutated, it upregulates the expression of β-hCG. As a result, it allows for β-hCG to suppress immune responses through downregulating IL3, IL13R, TNF12, and TNF10 [61,108,109]. It also has been shown that the mutated BRCA1 induces β-hCG-mediated tumorigenesis through TGFBR1 signaling [61]. Therefore, Sengodan et al. found that BRCA1 increases the expression of β-hCG, which is a molecule that induces metastasis of breast and ovarian cancer cells [61].

Transcriptional Regulation of Wild-Type BRCA1

There are also transcription factors that regulate the expression of BRCA1. The protein inhibitor of differentiation 4 (ID4) has been shown to downregulate the expression of BRCA1 in MCF-7 cells [68]. It was also found that the GA-binding protein (GABP)-α/β binds to the promoter and activates BRCA1 gene expression in MCF-7 cells and T47-D cells [68]. Additionally, the carboxyl-terminal binding protein 1 (CtBP1) has been found to be present in 92% of invasive breast cancer cases [110]. Through the use of nuclear staining of human breast tissue, transfections, and ChIP assays, Deng et al. found that CtBP1 downregulated BRCA1 at the transcriptional level [110]. It inhibits BRCA1 by binding to the promoter region, which enables CtBP1 to interact with DNA-binding proteins and co-repressor complexes such as CtIP [110]. As a result, BRCA1 mRNA levels are lower in cells with higher CtBP1 levels, which results in tumorigenesis [110].

There is also regulation of BRCA1 gene expression by the Rb-E2F pathway in murine and human cancers [111]. In BRCA1, there is a DNA-binding site for E2F directly up-stream of exon 1, and when E2F binds to this site, it transcriptionally activates the BRCA1
pathway [111,112]. However, Rb—a product of the retinoblastoma susceptibility gene (RB1)—inhibits the expression of E2F by binding to and blocking the activation domain of E2F proteins [111,113–115]. Therefore, when Rb inhibits the expression of E2F, it can no longer activate BRCA1 transcription, resulting in decreased BRCA1 gene expression [111]. This inactivation of the tumor suppressor gene contributes to cancer development [111]. These results were found through the use of in vivo transfections of E2F1 in transgenic mouse models, Northern blot analyses, electrophoretic mobility shift assays (EMSA), and plasmid and luciferase reporter assays [111].

Epigenetic Regulation of Wild-Type BRCA1

There are also epigenetic factors that regulate the expression of BRCA1 [68]. In one study, the effects of methylation on the promoter region of BRCA1 were investigated [68,116]. The methylation of the promoter caused the promoter to be less accessible [68,117]. As a result, the inability of activators to bind to their respective sites on the promoter leads to decreased BRCA1 transcription and gene expression [68,116]. Rice et al. conducted this experiment using high-resolution bisulfite sequence analysis of 21 axillary node-negative breast cancer patient specimens [116].

Lu et al. used a candidate gene approach to study how hypermethylation affects BRCA1 gene expression [117]. It was found that BRCA1 has a CpG island in its 5′ region of the promoter [117]. When this CpG island is hypermethylated, it results in the silencing of the expression of the BRCA1 gene [117–119].

The effects of hypoxia on BRCA1 gene expression because hypoxia often occurs in tumor microenvironments were investigated [120]. Specifically, Lu et al. examined the histone modifications that were affected by hypoxia [120]. Using ChIP assays and reporter constructs, Lu et al. analyzed the cells MCF-7, A549, RKO, and HCC 38 [120]. Lu et al. found that hypoxia increased H3K9 methylation and decreased H3K9 acetylation at the promoter region of BRCA1 [120]. Therefore, hypoxia can regulate BRCA1 expression by transcriptionally repressing the promoter through histone methylation and acetylation [120].

Bosviel et al. found that the metabolite S-equol had an effect on the gene expression of BRCA1 and BRCA2 in MCF-7 and MDA-MB-231 cells [121]. This resulted in the transcriptional activation of BRCA1 and BRCA2, increasing gene expression and tumor suppressor function [121]. As a result, demethylation activates gene expression in BRCA1 and BRCA2 [121].

Post-Transcriptional Regulation of BRCA1

There are miRNAs that regulate BRCA1 expression in triple-negative sporadic breast cancer cases [122]. Specifically, miR-146a and miR-146b-5p are two miRNAs that bind to the same site in the BRCA1 promoter region of the mRNA [122]. Using reporter assays and transfection of miRNAs in mammary cell lines, it was found that miR-146a and miR-146b-5p both downregulate the expression of BRCA1 [122]. Garcia et al. concluded this because they found that the miRNAs increased proliferation and decreased homologous recombination, which are two impaired processes that are normally regulated by BRCA1 [122].

Other post-transcriptional factors include the miR-15/107 group of miRNAs, which regulates the BRCA1 coding sequence in primates and rodents [123,124]. In this study, the miR-15/107 group of miRNAs were transfected into cell lines HT-29 and MCF-7 [124]. After using transient transfection, Quann et al. used quantitative reverse transcriptase PCR, luciferase validation of miRNA targets, Western immunoblotting, and statistical analyses to analyze the effects of the miRNAs on BRCA1 gene expression [124]. Eight of ten miRNAs in the miR-15/107 group downregulate BRCA1 mRNA abundance [124,125]. The miRNAs repress BRCA1 by degrading its RNA targets and repressing the translation of messenger RNAs (mRNAs) [124,126,127]. As a result, the miR-15/107 group of miRNAs is a repressor of BRCA1 gene expression through its coding sequence [124].
2.1.3. BRCA2

BRCA2 is a large protein made of 27 exons and 3418 acids that is localized to the nucleus in MCF7 cells [128–130]. There have been studies that have shown that BRCA2 may play a role in the regulation of transcription [129,131]. There has also been a study that has shown that BRCA2 is involved in DNA repair and recombination by binding to rad51 [129,132]. Bertwistle et al. found that BRCA2 is cell cycle-regulated and is induced at the late G1/early S phase, which is before DNA synthesis [129,133]. However, since BRCA2 is also involved in DNA repair, this means that during DNA synthesis its role could solely be to maintain genome integrity during replication [129,133]. Hence, BRCA2 is a tumor suppressor gene like BRCA1 [129].

Transcriptional Regulation of BRCA2

Mutations of BRCA2 have been linked to tumorigenesis in murine sporadic breast cancers [134,135]. In the murine BRCA2 gene, there is a region 148 bp upstream of the first exon that is necessary to activate transcription of BRCA2 in mammary cells [135,136]. It was also found that there is a 52 bp fragment between regions −92 and −40 bp that is necessary for promoter activity [136]. This is because it contains a CREB/ATF-binding site, where the CREB transcription factor family can bind to this region in the promoter to activate gene transcription [136]. Specifically, the transcription factors CREB-1, ATF-1, and CREM bind to their binding site on BRCA2 to activate gene expression [136]. Callens et al. inhibited these transcription factors, where a decrease in BRCA2 gene expression was observed [136]. Therefore, the CREB family upregulates BRCA2 gene expression [136].

There are DNA-damaging agents that regulate BRCA2 promoter activity in breast cancer cell lines [137]. Specifically, Wu et al. focused on the effects of adriamycin (ADR) and mitomycin C (MMC) on BRCA2 promoter activity [137]. Wu et al. found that ADR downregulates BRCA2 in a p53-dependent manner [137]. This means that the presence of ADR and MMC results in p53 inhibiting the USF transcription factor from binding to the BRCA2 minimal promoter, resulting in the downregulation of BRCA2 promoter activity [137]. This downregulation decreases BRCA2 mRNA and protein levels in the cell as a result of DNA damage induced by agents such as MMC and ADR [137].

Epigenetic Regulation of BRCA2

There are also epigenetic factors that regulate BRCA1 and BRCA2, such as hypermethylation [138]. Lobanova et al. used molecular genetic studies of 50 breast cancer tissues, each in different stages [138]. In 34% of the breast cancer cases, the promoter region of BRCA1 was hypermethylated [138]. The BRCA2 promoter region was hypermethylated in 50% of their cases [138]. In this case, hypermethylation inhibited BRCA1 and BRCA2 expression by blocking transcription factors from binding to the promoter [117–119]. Therefore, the promoter cannot be activated and mRNA and protein levels decrease [138]. This inhibits the function of BRCA1 and BRCA2 as tumor suppressor genes that regulate the cell cycle [130]. As a result, hypermethylation of BRCA1 and BRCA2 promoters can lead to the tumorigenesis of cells [138].

Dworkin et al. identified that methylation of BRCA2 can also affect BRCA2 gene expression [139]. In this study, 15 tumors that lacked the loss of heterozygosity (LOH) of the BRCA2 wild-type allele were analyzed [139]. Through sampling these tissues and analyzing their mutation type, it was found that silencing of BRCA2 through methylation was not very common in tumor cells [139]. However, in the tumor cells where BRCA2 was silenced, methylation silenced BRCA2 by binding to the promoter region and inhibiting other transcription factors from binding, like hypermethylation [139].

Post Transcriptional Regulation of BRCA2

There are also post-transcriptional factors that regulate BRCA2 expression through interactions with miRNAs [140]. Specifically, one study by Mogilyansky et al. tested the interactions of the miR-17/92 cluster with the mRNA of BRCA2 in pancreatic, breast, colon,
and kidney tissue cell lines [140]. Luciferase reporter assays were used to find that in the cluster, miR-19a and miR-19b interact with the 3′UTR region of BRCA2’s mRNA [140]. The overexpression of these miRNAs resulted in a decrease in BRCA2 mRNA levels and therefore a decrease in BRCA2 protein levels in these cell lines [140]. Therefore, miR-19a and miR-19b both downregulate BRCA2 gene expression by directly decreasing mRNAs [140].

There are also lncRNAs that are involved in regulating the repairment of double-stranded DNA breaks, which can promote tumorigenesis [141]. One known lncRNA is PCAT-1, which is a cytoplasmic lncRNA that is induced by genotoxic stress [141–143]. Presner et al. found that PCAT-1 regulates the expression of BRCA2 in prostate cancer cells [141]. PCAT-1 post-transcriptionally represses the BRCA2 3′UTR region of the mRNA, which disrupts homologous recombination (HR) [142].

3. iNOS/NO
3.1. General

Inducible nitric oxide synthase (iNOS, NOS2) is an enzyme that catalyzes the production of large amounts of nitric oxide (NO) by L-arginine [144,145]. Specifically, it oxidizes L-arginine to produce L-citrulline and NO [146,147] (Figure 1). L-Arginine uptake and availability are controlled by the cationic amino acid transporters CAT1, CAT2, and CAT3 [148]. These transporters control L-arginine uptake, which in turn regulates the production of NO by iNOS [148]. iNOS is a main source of NO in the body and regulates the immune system [149,150].

![Figure 1. NOS enzymatic reaction. The chemical reaction catalyzed by all the three human NOS enzymes involves the formation of L-citrulline and NO from L-arginine and in presence of O2 and NADPH. The three known human NOS genes are: NOS1 or neuronal NOS (nNOS), NOS2 or inducible NOS (iNOS), and NOS3 or endothelial NOS (eNOS).](image-url)

Unlike eNOS and nNOS, iNOS is not regulated by calcium levels in the body [150–152] (Figure 1). In order to activate eNOS and nNOS expression, calcium (Ca2+) has to bind to calmodulin (CaM) to allow CaM to bind to the CaM-binding domains of eNOS and nNOS [153,154]. However, in iNOS, CaM is naturally tightly bound to the CaM-binding domain without the need for Ca2+ [147,155]. As a result, iNOS is unregulated by intracellular Ca2+ levels and therefore is a major contributor to the overproduction of NO in the body [140,150]. The positive effects of NO overproduction include defending the host against viral or microbial pathogens. In the Vaccinia virus, NO inhibits DNA synthesis by inhibiting the activity of DNA-synthesizing enzymes such as ribonucleotide reductase (RR) [156,157]. NO inhibits the activity of RR by scavenging the tyrosyl radical that is necessary for the catalysis of RR [158]. As a result, there are decreased levels of Vaccinia
protein synthesis. This was found by generating the Vaccinia virus recombinant that expressed murine iNOS in vitro [157,159,160]. The negative effects include contributing to the pathogenesis of inflammatory diseases such as atherosclerosis [149,150,161–163]. iNOS is expressed in atherosclerotic lesions, where NO reacts with superoxide anion, causing oxidative damage that leads to cellular damage and inflammation [162,164,165].

There are different functions of NO synthesized by iNOS, where the concentration level of NO can either protect against disease or contribute to the pathogenesis of disease [166–168]. iNOS producing NO can have immunoregulatory effects by functioning as an intra- and intercellular signaling molecule that can inhibit or enhance the immune response [149,160,169]. NO binds to and induces a conformational change in the enzymes involved in immune responses. The conformational change can either activate the enzyme or hinder the ability of the enzyme to catalyze the reaction [149]. However, the expression of iNOS is also involved in the pathogenesis of immune diseases. This is seen in the L. donovani infection in the liver, where the expression of iNOS inhibits T cell proliferation or induces T cell apoptosis, furthering the progression of infection [149,169–171].

3.1.1. Transcriptional Regulation of iNOS

The human iNOS gene is located on chromosome 17 and consists of 26 exons and 25 introns [172,173]. iNOS gene expression is regulated at different levels, including transcriptional regulation [173,174]. A number of transcription factors were reported to bind the iNOS gene promoter, resulting in either the activation or the inhibition of iNOS transcription [169,175].

In Table 1, one of the first transcription factors identified as a direct transcriptional inducer of the iNOS gene is the nuclear factor (NF)-κB [169,176]. In addition, iNOS transcription was found to be under the regulation of the STAT-1α pathway. When IFN-γ is present in the cell, it activates cytoplasmic JAKS that tyrosine phosphorylates the transcription factor STAT-1α. This process enables STAT-1α to dimerize, translocate to the nucleus, and activate iNOS gene expression [169,177–179].

### Table 1. Regulation of iNOS.

| Transcriptional Factors: Name                  | Type of Factor                  | Expression                      |
|-----------------------------------------------|--------------------------------|---------------------------------|
| NF-kB                                         | Pre-transcriptional factor      | Upregulation                    |
| STAT-1α                                       | Pre-transcriptional factor      | Upregulation                    |
| cAMP-induced transcription factors           | Pre-transcriptional factors     |                               |
| AP-1                                          | Pre-transcriptional factors     | Downregulation in human iNOS    |
| Octamer factor                                | Pre-transcriptional factors     | Upregulation                    |
| PPAR                                          | Pre-transcriptional factors     | Downregulation                  |
| p53                                           | Pre-transcriptional factors     | Downregulation                  |
| HIF-1                                         | Pre-transcriptional factors     | Upregulation                    |
| RAR-α                                         | Pre-transcriptional factors     | Downregulation                  |
| ER-β                                          | Pre-transcriptional factors     | Upregulation                    |
| Nostrill (lncRNA)                             | Transcription factor            | Upregulation                    |
| DNA methylation                               | Epigenetic post-transcriptional regulation | Downregulation |
| Histone H3K9 methylation                      | Epigenetic post-transcriptional regulation | Downregulation |
| Hypermethylation                              | Epigenetic post-transcriptional regulation | Downregulation |
| Demethylation                                 | Epigenetic post-transcriptional regulation | Upregulation |

The cAMP-induced transcription factors bind to the C/EBP-binding sites at positions −155 to −163 bp on the murine iNOS promoter [180]. In addition, AP-1 is a transcription factor that regulates murine iNOS expression by binding to the iNOS promoter at position −1125 bp. However, in human iNOS, overexpression of AP-1 was shown to inhibit iNOS promoter activity using a supershift assay [169,175].

The octamer factor binds to the iNOS promoter region 24 bp upstream from the TATA box and activates iNOS transcription in murine and rat cells [169,181–183]. The peroxisome
proliferator-activated receptors (PPARs) inhibit iNOS promoter activity indirectly by inhibition of NF-κB or AP-1 through competition for CBP/p300 in human chondrocytes and murine macrophage cells [169,184–186].

The tumor suppressor p53 inhibits iNOS promoter activity through transcriptional trans-repression of the promoter in human and murine epithelial cells [169,187,188]. The hypoxia-induced factor-1 (HIF-1) enhances iNOS promoter activity by binding to the HIF-1 site on the promoter in murine macrophages [169,189,190]. The retinoic acid receptor-α (RAR-α) uses RXR-PPAR-γ heterodimers to inhibit murine iNOS activity [169,191–193]. The estrogen receptor-β (ER-β) mediates the transcriptional activation of the murine iNOS promoter, which was found using an immunoblot analysis [169,193,194].

Long non-coding RNAs (lncRNAs) were further validated to control iNOS gene expression. Specifically, the effects of the intergenic lncRNA named Nostrill on iNOS gene expression were analyzed in human microglia [195]. When Nostrill expression is induced by cytokine LPS [195–198], it drives secondary and late iNOS gene transcription [195,199,200]. It can also scaffold the RNA polymerase II at the iNOS promoter region, enhancing the efficiency of the promoter, ultimately increasing iNOS transcription [195,201–203].

3.1.2. Epigenetic Regulation

The human iNOS gene is also regulated at an epigenetic level. It was found that DNA methylation played a large role in the transcriptional silencing of the iNOS gene at its promoter [204,205].

Acetylation is another epigenetic factor that regulates iNOS gene expression [206]. Hypermethylation of the iNOS gene promoter can regulate iNOS gene expression [207]. Demethylation is another epigenetic factor that can regulate the expression of the iNOS gene in human articular cartilage cells [208]. To study the effects of demethylation, one study used transfection of human articular cartilage samples and fluorescence-activated sorting [208] (Table 1).

3.1.3. Post-Transcriptional Regulation

iNOS expression is further regulated at a post-transcriptional level mainly through destabilization of its transcript [209]. Furthermore, several microRNAs have been reported to negatively regulate iNOS gene expression, directly or indirectly through facilitating mRNA degradation or inhibiting translational activity and protein synthesis (Table 1).

3.1.4. iNOS-Derived NO Levels in Cells

NO is a signaling molecule that is produced by NO synthases eNOS, nNOS, and iNOS [146,147]. Specifically, iNOS is responsible for the regulation of NO in the immune system [149,150]. iNOS transcription is induced by cytokines such as LPS and IFN-γ [210,211]. One experiment used the direct measurement of NO release using an NO-specific amperometric probe and a cyclic AMP assay [211]. They found that low NO basal levels are always present in the cytoplasm of the cell [210–212]. It also takes 3–4 h for NO levels to be increased by iNOS induction [210–212]. They conducted a 48 h experiment, where they found that iNOS is capable of producing and maintaining higher NO levels for 24–48 h after induction before returning to basal levels [210].

After NO is produced by iNOS, it has a half-life in the range of 9–900 min [213,214]. This half-life is a result of physiological NO reacting with oxygen (O2) to produce nitrite (NO2-) [215]. Nitrite is then further oxidized to form nitrate (NO3-), where NO is metabolized [215]. This results in the return of NO levels in immune cells to return to basal levels [215]. In biological systems, the metabolic rate of NO to nitrite and nitrate is dependent on both oxygen concentrations and ambient NO concentrations [215]. As a result, the shortest half-life of NO occurs after iNOS is induced in immune cells, where NO concentrations are at the highest levels [210,214].
3.2. iNOS-Induced NO Effects

The induction of iNOS increases levels of exogenous NO, which can lead to the S-nitrosylation of different transcription factors. S-nitrosylation is the process of covalently attaching a nitric oxide moiety to a cysteine thiol. This process can result in regulating the function and expression of different proteins [216,217].

Reynaert et al. investigated the effects of iNOS-induced NO S-nitrosylation of the NF-kB family of transcription factors [217,218]. NF-kB transcription factors play a large role in immune and inflammatory responses [217]. It also regulates cell survival and proliferation [174]. S-nitrosylation by NO in NF-kB inhibits NF-kB-dependent gene transcription, promoter activity, and DNA binding [217,219,220].

3.3. iNOS/NO Functions in Cancers

iNOS-induced NO plays many roles in cancer development [16]. One study found that iNOS-induced NO upregulates the expression of matrix metalloproteinase 2 (MMP2), MMP-0, and VEGF, which promote metastasis [16].

Kielbik et al. found that iNOS-induced NO can also lead to the progression of ovarian cancer [4,221,222]. NO suppresses BRCA1 and BRCA2 promoter activity, which decreases their mRNA expression [4]. BRCA2 is another tumor suppressor gene that is involved in DNA repair [129].

Saed et al. analyzed epithelial ovarian carcinoma (EOC) cells in their pro-oxidant state [219–226]. In this state, there is increased expression of both iNOS and NO, and when iNOS was induced by L-arginine, it resulted in lower apoptosis in the EOC cells [223–226]. The mechanism behind this was that iNOS-induced NO S-nitrosylated caspase-3. Caspase-3 is a lysosomal enzyme that is involved in apoptosis for the cells, where S-nitrosylation decreases its activity and therefore decreases apoptosis in EOC cells, which results in the progression of ovarian cancer [223–226].

Sha and Marshall investigated how iNOS-derived NO-dependent S-nitrosylation post-transcriptionally modified proteins [227]. The effects on the p53 tumor suppressor protein were studied [227]. p53 is modified by Hdm2-mediated proteasomal degradation [227,228]. However, when NO is present, it S-nitrosylates Hdm2, inhibiting the Hdm2 pathway [227,229]. Therefore, it increases p53 levels in the cell through S-nitrosylation of Hdm2, which is a ubiquitin ligase [227,229]. It has also been found that S-nitrosylation of the protein S100B can increase p53 activity because S100B is a binding partner of p53 [227,228].

Jia et al. investigated how iNOS-induced NO can S-nitrosylate GAPDH, which is a key glycolytic enzyme [230,231]. When GAPDH is S-nitrosylated, it regulates enzyme activity [230,231]. iNOS induces the S-nitrosylation of GAPDH at Cys152 or Cys247 in response to the activation of the S100A8/A9 complex [231]. Additionally, it was found that the S-nitrosylation of GAPDH dysregulates the IFN-γ translational pathway, which is a pathway that exhibits anti-tumor properties [231,232]. As a result, iNOS-induced NO S-nitrosylation can regulate the expression of target proteins that can result in inflammation and cytotoxicity within different cells [231].

4. iNOS and Breast Cancer Implications

4.1. Triple-Negative Breast Cancer (TNBC)

iNOS-induced NO has been shown to contribute to the progression of basal-like triple-negative breast cancer (TNBC) [233,234]. NO induced mutations in p53 and activated the epidermal growth factor receptor (EGFR) through S-nitrosylation [234–237]. Specifically, this S-nitrosylation resulted in the phosphorylation and therefore activation of the EGFR/ERK/MAPK pathway [14,236]. When this pathway is phosphorylated, it activates the metabolite prostaglandin E2 (PGE2) [14,238,239]. PGE2 was found to promote cell migration, invasion, and proliferation on top of inhibiting apoptosis [14,238,239]. PGE2 also leads to COX-2 overexpression, which promotes inflammation in cells [233,236]. This is important because inflammation is a major driver of cancer metastasis [240]. These results are schematically represented in Figure 2.
nitrosylation can regulate the expression of target proteins that can result in inflammation and cytotoxicity within different cells [231].

4. iNOS and Breast Cancer Implications

4.1. Triple-Negative Breast Cancer (TNBC)

iNOS-induced NO has been shown to contribute to the progression of basal-like triple-negative breast cancer (TNBC) [233,234]. NO induced mutations in p53 and activated the epidermal growth factor receptor (EGFR) through S-nitrosylation [234–237]. Specifically, this S-nitrosylation resulted in the phosphorylation and therefore activation of the EGFR/ERK/MAPK pathway [14,236]. When this pathway is phosphorylated, it activates the metabolite prostaglandin E2 (PGE2) [14,238,239]. PGE2 was found to promote cell migration, invasion, and proliferation on top of inhibiting apoptosis [14,238,239]. PGE2 also leads to COX-2 overexpression, which promotes inflammation in cells [233,236]. This is important because inflammation is a major driver of cancer metastasis [240]. These results are schematically represented in Figure 2.

Figure 2. Role of NO in breast cancer (BC) and other relevant cancers. (A) Pathways regulated by NO in BC and relative intracellular NO-mediated effects. (B) Direct (plain lines) and indirect (dotted lines) pathways correlating NO with HER2 (top), (C) BRCA1 (middle), and (D) BRCA2 (bottom). OC, ovarian cancer; PC, pancreatic cancer; EMT, epithelial–mesenchymal transition.

In TNBC, Garrido et al. found that iNOS-induced NO activated the NF-kB pathway and increased the secretion of cytokines IL-8, IL-1β, and TNFα [14,241–243]. Interestingly, the activation of the EGFR pathway induced the expression of these cytokines [14]. IL-8 has been shown to cause cell invasion, metastasis, and epithelial-to-mesenchymal transition [14,236,244]. IL-1β and TNFα have cytotoxic effects in the cell and promote tumor progression [14,245–248]. NF-kB promotes inflammation in cancer cells [50,53]. Overall, iNOS actsives the EGFR/ERK/MAPK pathway, which results in the activation of PGE2, COX-2, IL-8, IL-1β, TNFα, and NF-kB [14]. All of these factors promote the progression of cancer through metastasis, cell invasion, and inflammation [14].

iNOS expression regulates the expression of TNBC biomarkers [15]. Chen et al. used shRNA-guided knockdown to downregulate iNOS in TNBC cells [15]. When iNOS was knocked down, tumor marker CD1 along with special TNBC biomarkers RUNX1 and BCL11A were downregulated [15]. The iNOS knockdown also upregulated the tumor suppressor CK1 [15]. As a result, the knockdown of iNOS can partially reverse the tumorigenesis of TNBC cells [15].

Chen et al. found similar effects when iNOS was inhibited by NG-monomethyl-L-arginine monooacetate (L-NMMA) and 1400 W [15,249]. The inhibition of iNOS decreased cell proliferation, cell migration, and cancer stem cell self-renewal [15,249]. iNOS overexpression can also result in colon adenoma, enhanced KRAS-induced lung carcinogenesis, inflammation, tumor growth, and glioma stem cell proliferation [15,188,250,251].
4.2. ER Breast Cancer

In estrogen receptor-negative (ER-) breast cancer, it was found that Ets-1 is a transcriptional mediator of NO signaling [16,243]. Ets-1 promotes tumorigenesis by activating the Ras/MEK/ERK signaling pathway [16,237]. Ets-1 is a proto-oncogene that promotes angiogenesis and extracellular matrix remodeling [16,252–254]. It is activated through binding to the MMP-9 gene [16,255]. MMP-9 expression also promotes HER2 oncogenic expression [16,256,257]. As a result, when Ets-1 is activated, it binds to and activates MMP-9 expression, which in turn also activates HER2 [16,255–257]. Therefore, there is an indirect relationship between NO and HER2 as a result of them both being mediated by the transcription factor Ets-1 [16]. These results are shown above in Figure 2B.

Mishra et al. analyzed the effects of eNOS-induced NO on the progression of cancer [16]. In the presence of estrogen, eNOS activates the phosphatidylinositol 3-kinase (PI3K)/Akt/eNOS signaling pathway [16,258,259]. It also activates the ERK-1/2 pathway [16,258,259]. Both of these pathways have signaling-mediated effects that promote breast cancer [16,258,259]. It was also found that in the breast cancer subtype invasive ductal carcinoma, NO biosynthesis was upregulated in higher-grade tumors [16,17].

5. iNOS and Breast Cancer Oncogenes HER2, BRCA1, and BRCA2

iNOS-derived NO has been found to contribute to the progression of breast cancer [12,13]. Specifically, iNOS-derived NO can disrupt DNA repair mechanisms and can cause genomic instability [13,260–263]. It has been found that the rise in NO levels can alter the levels of cell proliferation and apoptosis in cells [13,234,264]. This can lead to mutations that are linked to the initiation, promotion, and progression of cancer [13].

5.1. iNOS and HER2

The relationship between IFN-γ, iNOS, and HER2 expression was investigated by Marth et al. [228]. IFN-γ induces the expression of iNOS [265]. However, IFN-γ decreases the expression of the HER2 oncogene [265,266]. This was against the predictions of the study because iNOS is often related to the induction of oncogenes [265]. Marth et al. suspected that this lack of correlation could be due to the fact that iNOS is dependent on the activation of other signaling pathways besides IFN-γ [265,267,268]. Therefore, other signaling pathways could be necessary to establish the relationship between iNOS and HER2 [265,267,268].

5.2. iNOS and BRCA1

Yakovlev et al. analyzed how iNOS affects the function of BRCA1 in breast cancer cells [13,269]. BRCA1 is a tumor suppressor gene that is involved in cell cycle regulation and DNA repair [71–73]. The presence of iNOS-induced NO led to the dephosphorylation of RBL2 in the promoter region of BRCA1 [13,270,271]. This causes the formation of the RBL2/E2F4 complex in the same region [13,270,271]. As a result, E2F4 replaces E2F1 in binding to the BRCA1 promoter [13,270,271]. It is important because E2F1 is an activator while E2F4 is an inhibitor, where this shift causes the inhibition of BRCA1 promoter activity [13,270,271]. These results are shown above in Figure 2C.

Therefore, BRCA1 cannot perform homologous recombination repair, resulting in the increase in error-prone nonhomologous end joining (NHEJ) in the cell [13]. The increase in mutations causes inflammation, carcinogenesis, and genomic instability, which all contribute to the progression of breast cancer [13]. Yakovlev et al. came to these conclusions by performing cell cultures with MCF-10A, AF49, and RAW264.7 cells [13].

Plenchette et al. found that iNOS-induced NO can alter BRCA1 tumor suppressor activity through NO donors [13,272]. Specifically, the NO donors S-nitroso-N-acetylpenicillamine (SNAP) and diethylenetriamine NONOate (DETANONOate) have been found to promote the inhibition of BRCA1 expression [13,272]. This results in the hindrance of the ability of BRCA1 to repair DNA through the HR pathway, which leads to NHEJ and tumorigenesis [13,272].
5.3. iNOS and BRCA2

Garcea et al. found that iNOS-induced NO contributed to the inhibition of 8-OH-dg adducts [1,273]. This inhibition results in the promotion of pancreatic cancer cell growth [1,273]. However, BRCA1 and BRCA2 contribute to the repair of these same 8-OH-dg adducts [1,274]. Therefore, iNOS-induced NO indirectly suppresses BRCA1 and BRCA2 tumor suppressor functions [1,274]. Kielbik et al. also found that iNOS directly inhibits the promoter activity of BRCA1 and BRCA2 in ovarian cancer cells. As a result, this decreases the expression and function of BRCA1 and BRCA2 [4].

The relationship between iNOS and BRCA2 was found in ovarian and pancreatic cancer cell lines. However, little is known about the relationship between iNOS and BRCA2 in breast cancer cell lines. Therefore, further research is necessary to investigate whether or not the relationship between iNOS and BRCA2 in breast cancer cell lines follows the same trends as that of pancreatic and ovarian cancer cell lines. These results are shown above in Figure 2D.

6. Implication of iNOS, HER2, and BRCA1/2 in CSC Pathophysiology

Cancer stem cells (CSCs) constitute a small fraction of cancer cells within the tumor bulk that possesses pluripotent and renewing properties [275]. CSCs are thought to be responsible for driving oncogenesis, disease progression, metastasis, and therapeutic resistance [275]. Recent findings have confirmed the contribution of NO metabolism in defining the “stemness” properties of CSCs through cross-regulation of “stemness” associated signaling pathways, including the Notch and Wnt cascades [276]. Therefore, molecules able to modify the maintenance of a stem cell phenotype, including NO, are of great research and therapeutic interest.

Although initial reports have demonstrated that iNOS-generated NO is a distinctive feature of CSCs originated mainly by tumors developed in an inflammatory background, further studies demonstrate differential expression and function of NOS isoforms in CSCs that mainly depend on tumor type and aggressiveness. For example, in hepatocellular carcinoma, iNOS (NOS2) overexpression in cancer cell lines and human tissues promotes NOTCH-1-mediated stemness and tumor initiation in vivo, through a cGMP/PKG dependent mechanism [277]. Treatment of lung cancer cells with NO concentrations ranging between 20 and 40 µM, which are similar to those produced by iNOS, was able to induce dedifferentiation of lung cancer cells towards a stem-cell-like phenotype through stabilization of critical CSC-associated markers, such as Oct4 [278]. Accordingly, selective inhibition of the high endogenous iNOS expression in TNBC cells significantly reduced CSC self-renewal ability, tumor initiation, and the number of lung metastases as a result of EMT inhibition [279]. Furthermore, targeting of endogenous NO production by iNOS silencing in ER+ breast cancer cells displayed inhibition of mammosphere formation and expression of CSC-associated markers, while it significantly reversed tumor resistance to tamoxifen treatment [280].

Our preliminary findings obtained by gene microarray analysis of a CD133+/CD44+ CSC population isolated from the pancreatic adenocarcinoma (PDAC) cell lines PANC1 and MiaPACA2 revealed a strong iNOS mRNA induction in MiaPaca2-derived CSCs compared to non-SC population (CD133-/CD44-), whereas in PANC1 CSCs, iNOS was significantly inhibited (Figure 3). Furthermore, iNOS overexpression in MiaPaca2 CSCs was positively correlated with a concurrent significant increase in the mRNA expression of reported co- and trans-activators of iNOS transcription, including AP-1, CEBPB, GATA 1, NFAT5, NFATC4, NF-κB, STAT-1a, TP53, and IL-1β (Figure 3). Respectively, iNOS downregulation in PANC1 CSCs was associated with a reduction in the mRNA levels of GATA 1, NF-κB, Sp1, and CREB (Figure 3). The observed difference in the iNOS expression profiles between the two cell lines may be attributed to the diversity of tumor aggressiveness, with MiaPaca2 depending more on high iNOS levels in order to sustain the aggressive phenotype [281].
The above findings indicate that the type and expression levels of each NOS isoform in pathways, has been highlighted in several reports [284]. A splice variant of full-length HER2 isoforms with significant implications in the regulation of HER2 + breast CSC features, including tumor initiation, EMT induction, and resistance to targeted therapy [284]. However, no direct associations between iNOS, BRCA1, and HER2 expression profiles have been established so far in any type of CSCs, including breast CSCs.

In contrast, colon cancer mesenchymal cells do not express iNOS and instead over-express eNOS that impairs the CSC phenotype and induces tumor cell proliferation [282]. The above findings indicate that the type and expression levels of each NOS isoform in CSCs might be specific to cancer type and tumor aggressive potential, as well as the impact of inflammation-induced iNOS and NO in tumorigenesis. In addition, more precise studies on purified CSC populations are needed for drawing safer conclusions on iNOS impact on cancer “stemness”.

Downregulation of BRCA1 has been associated with a significant increase in the CSC-like populations in breast cancer cells, whereas BRCA1 reconstitution increases cell sensitivity to HDAC inhibitor-induced loss of stemness, thus suggesting that BRCA1 functions as a breast stem cell regulator, while its status may determine tumor response to therapy [283]. Likewise, the key role of altered (overexpressed/amplified) HER2 signaling in the maintenance and enrichment of breast CSCs, through crosstalk with stemness-related pathways, has been highlighted in several reports [284]. A splice variant of full-length HER2 mRNA and a collection of HER2 truncated carboxy-terminal fragments, known as d16HER2 and p95HER2, respectively, have been characterized as the most oncogenic HER2 isoforms with significant implications in the regulation of HER2+ breast CSC features, including tumor initiation, EMT induction, and resistance to targeted therapy [284]. However, given the direct implication of BRCA1/2 signaling in the pathophysiology of hereditary pancreatic adenocarcinoma (PDAC) [285], as well as the recently reported prognostic impact of HER2 expression or amplification in the survival of PDAC patients [286], we examined possible alterations in the expression profiles of BRCA1/2 and HER2 mRNAs in our PDAC CSC models. Both MiaPaca2 and PANC1 cell lines are proficient in BRCA1/2 wild-type expression [287], while HER2 levels are more profound in MiaPaca2 than PANC1 [288].

Figure 3. mRNA differential expression of iNOS and iNOS-inducing genes in CD133+/CD44+ CSCs isolated by the PDAC cell lines MiaPaca2 and PANC1. For gene microarray analysis, Agilent Array platform was employed. Quantile normalization and subsequent raw data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). Differentially expressed mRNAs between compared samples (CD44+/CD133+ vs. CD44+/CD133-) of each cell line were identified through fold change (FC) filtering (FC ≥ 2 was set as a cut-off value). Columns represent mean fold change of gene expression ± SDEV.
CD133+/CD44+ CSCs from both cell lines significantly overexpress BRCA1 compared to the corresponding CD133+/CD44- non-SC populations, whereas HER2 overexpression was observed only in MiaPaca2-derived CSCs. In contrast, PANC-1-derived CSCs showed significant inhibition of HER2 mRNA expression (Figure 4). No significant differences in BRCA2 mRNA expression were detected in our CD133+/CD44+ enriched CSC populations from both cell lines. Overall, our findings from our PDAC CSC model suggest a positive correlation of iNOS, BRCA1, and HER2 expression in CSCs of aggressive tumors that may be critical for sustaining cell “stemness” and associated properties (Figure 4).

Figure 4. Differential expression of BRCA1, BRCA2, and HER2 mRNA transcripts in CD133+/CD44+ CSCs isolated by the PDAC cell lines MiaPaca2 and PANC1. For gene microarray analysis, Agilent Array platform was employed. Quantile normalization and subsequent raw data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). Differentially expressed mRNAs between compared samples (CD44+/CD133+ vs. CD44-/CD133-) of each cell line were identified through fold change (FC) filtering (FC ≥ 2 was set as a cut-off value). Columns represent mean fold change of gene expression ± SDEV.

7. Bioinformatic Analyses: Correlation between BRCA1/2 Mutations and Genes Involved in the NOS Pathway

Through the development of high-throughput technologies for the analysis of molecular alterations associated with tumor development, a huge amount of bioinformatics data has been generated and collected in publicly available databases [289]. Among these, The Cancer Genome Atlas (TCGA) consortium collects clinical information as well as gene expression, ncRNA expression, DNA methylation, and protein expression data of 33 different human cancers [290].

The analyses of these data have allowed the identification of novel potential diagnostic and prognostic biomarkers for different tumors through the identification of specific genes, miRNAs, or proteins dysregulated in cancer [291–293].

Despite the availability of all these molecular data also for breast cancer, only a few studies have filtered patients according to the presence of BRCA1/2 mutations or the amplification/overexpression of ErbB2. Similarly, no in-depth correlation studies have been performed between the expression levels of amplified or mutated genes such as ErbB2 and BRCA and the expression levels of genes involved in the NOS pathway.

To establish the correlation existing between genetic alterations affecting BRCA1, BRCA2, and ErbB2 with the expression levels of NOS-associated genes, the phenotype and gene expression data contained in the TCGA Breast Cancer (TCGA BRCA) database were evaluated. In particular, the TCGA BRCA database contains 24 different datasets collecting clinical and molecular information on a total of 1247 breast cancer patients [294].

The datasets “Phenotypes” and “IlluminaHiSeq Gene Expression RNAseq” were downloaded in order to identify breast cancer patients with mutations affecting BRCA1 or
BRCA2 or amplification affecting ErbB2 and the expression data of 20,530 different genes, respectively.

Through the “Phenotypes” dataset, 25 BRCA1 and 22 BRCA2 mutated patients were identified. Similarly, a total of 68 breast cancer patients with ErbB2 amplification were identified, of which 14 had a HER2 FISH ratio > 2.2 and 47 had a HER immunohistochemistry (IHC) score of 3+ or higher. Seven patients had both a HER2 FISH ratio > 2.2 and a 3+ IHC score. For these subsets of patients, the expression levels of BRCA1, BRCA2, and ErbB2 were obtained by analyzing the “IlluminaHiSeq Gene Expression RNAseq” dataset. From the same dataset, the expression levels involved in the NOS pathway were observed, including NOS1, NOS3, nitric oxide synthase interacting protein (NOSIP), and nitric oxide synthase 1 adaptor protein (NOS1AP). Of note, NOSIP negatively regulates the production of nitric oxide by inducing NOS1 and NOS3 translocation to the actin cytoskeleton, thus inhibiting their enzymatic activity [295], while NOS1AP is mainly involved in the modulation of neuronal NO through the regulation of NOS1 with other proteins [296]. Unfortunately, no expression data about NOS2 were recorded on the “IlluminaHiSeq Gene Expression RNAseq” dataset; therefore, this gene was not investigated (Figure 5).

Figure 5. Correlation analyses between the expression levels of ErbB2 and NOS genes in TCGA breast cancer patients with HER2 amplification. Each black dot represents a cancer patient.

Pearson’s correlation and Spearman’s correlation analyses were performed for the three groups of breast cancer patients, i.e., BRCA1-mutated, BRCA2-mutated, and ErbB2-amplified breast cancer patients, depending on the normal or non-normal distribution of expression data (Figure 6).
Figure 6. Correlation analyses between the expression levels of BRCA2 and NOS genes in TCGA breast cancer patients with mutations affecting BRCA2. Each black square represents a cancer patient.

The correlation analyses between the expression levels of BRCA1 and NOS genes revealed how the expression levels of dysregulated BRCA1 due to somatic mutations positively correlate with the expression levels of NOS1 ($r = 0.5191; p = 0.0078$), while no significant correlations were observed between BRCA1 and NOS3, NOSIP, and NOS1AP (Figure 7).

Figure 7. Correlation analyses between the expression levels of BRCA1 and NOS genes in TCGA breast cancer patients with mutations affecting BRCA1. Each black triangle represents a cancer patient.
8. Discussion and Perspectives

It has been demonstrated that human breast cancer tissues express high levels of iNOS. This expression has predicted increased tumor progression and a poor outcome of survival in women with estrogen receptor alpha-negative (ER-negative) tumors [193,297]. More recently, the same group of investigators reported that the co-expression of iNOS and COX2 enhances tumor growth and shortens the survival of patients with ER-negative breast cancer [298].

Several mechanisms have been suggested for the role of iNOS as a driver of breast cancer progression. For instance, high iNOS expression has been correlated with high p53 mutations. Additionally, iNOS-derived NO activates several survival signaling pathways, promotes HIF1-alpha stabilization (tumor cells cope with hypoxia), and mediates immuno-suppression and metastasis [298]. In TNBC, iNOS-induced NO resulted in mutations of p53 and activation of EGFR. This was through S-nitrosylation leading to the activation of the EGFR/ERK/MAPK and NF-kB pathways [16]. These activated pathways led to tumor cell proliferation, migration, invasion, and resistance to cytotoxic drugs [16].

Interestingly, the relationship between iNOS expression and the expression of breast cancer protooncogenes HER2, BRCA1, and BRCA2 in the pathogenesis of breast cancer is not well understood. Herein, we have discussed this relationship based on reported literature. In addition, we have also used bioinformatics to examine the possible linkage between the NOS pathway and the expression of the protooncogenes.

In ER-negative breast cancer, the protooncogene ETS-1 is a transcriptional mediator of NO and promotes angiogenesis. Activated Ets-1 binds and activates MMP9 expression, which in turn activates and increases the expression of HER2. Thus, a linkage is observed between NO and HER2. However, in other reports, it was found that while IFN-g induces the expression of iNOS, it also inhibits the expression of HER2 [298]. It is possible that depending on the stimulus, various signaling pathways may result in either the activation or inhibition of HER2 expression. It will be useful to ascertain the various conditions under which NO activates or inhibits HER2 expression in order to develop appropriate interventions that regulate iNOS expression.

Regarding the relationship between iNOS/NO and BRCA1, it was found that NO indirectly inhibits BRCA1 promoter activity, and NO can alter BRCA1 tumor suppressor activity. Similar findings were reported that NO suppresses the tumor suppressor activity of BRCA2 [13]. Clearly, iNOS-mediated NO affects the tumor suppressor activities of both BRCA1 and BRCA2 as well as potentiating the expression of HER2. These effects result in the promotion of tumor growth and malignancy. While many stimuli from the tumor microenvironment increase iNOS expression in normal cells, it is possible that such an increase is involved in the initial trigger of oncogenesis, alone or with other factors.

Several mechanisms have implicated the roles of NO and RNS in the induction of tumorigenesis. For instance, NO leads to oxidative nitrative stress that promotes DNA damage, suppression of DNA-repair enzymes, post-translational modification of proteins, and the formation of nitrosamines [11,299]. In addition, mutations in various genes have been reported to be strong genetic risk factors for ovarian cancer progression [4]. Interestingly, BRCA1 and BRCA2 mutations are responsible for the development of about 90% of all ovarian cancers [222]. Genes controlling cell growth, DNA repair processes, and apoptosis (e.g., TP53, BRCA1, BRCA2, and PARP) appear to be prime targets for mutations by NO/RNS.

Additionally, the expression of iNOS in breast CSCs is associated with malignancy and tumor growth. Its selective inhibition has been shown to reduce CSC self-renewal capacity [257]. In a recent study, Lopez-Sanchez et al. have investigated the resistance of ER-positive breast cancer CSCs to anti-hormonal therapy (tamoxifen) [280]. Lopez-Sanchez et al. examined the role of NO in CSC characteristics and examined whether targeting NO in breast CSCs would have any effect on sensitivity to tamoxifen. Silencing of NO or iNOS in CSCs resulted in the inhibition of mammospheres and CSC biomarker expression and sensitized the CSCs to tamoxifen-mediated cytotoxicity. These findings were consistent
with the findings that tamoxifen-resistant cells exhibited overexpression of iNOS and NOTCH-1 when compared to parental cells.

Kaplan–Meier survival analysis confirmed these in vitro findings. This analysis found that low NOS2 expression was associated with lowered metastasis in ER+ breast cancer patients that were treated with tamoxifen. Clearly, both tumor type and iNOS expression levels in CSCs dictate the aggressiveness and malignancy of cancer cells. Thus, targeting iNOS is a therapeutic strategy to treat breast cancer, alone or in combination with cytotoxic drugs [300]. Of interest, the expression of BRCA1 in breast cancer inversely correlates with the frequency of the CSCs [301]. In contrast, overexpression of HER2 correlates with enrichment of CSCs [284]. It will be useful to investigate the associations of iNOS, BRCA1, and HER2 expression in CSCs.

Similar findings to those observed in breast cancer were also found in bladder cancer CSCs [302]. In bladder cancer CSCs, high expression of iNOS was associated with increased invasion and tumor recurrence. Additionally, inhibition of iNOS inhibited tumor progression and reduced the number of CSCs. Therefore, the findings demonstrated that iNOS plays a central role in bladder cancer progression and the maintenance of CSCs.

Bioinformatic analyses did not reveal the correlations between iNOS, mutated BRCA1/2, and overexpression of HER2 in breast cancer datasets. However, the expression levels of gene products in the NOS pathway were able to be detected, namely NOS1, NOS3, NOSIP, and NOS1AP. The expression levels of mutated BRCA1 correlated with the expression of NOS1 with no significant correlations with NOS3, NOSIP, and NOS1AP.

Noteworthy, several studies have already demonstrated the pivotal role of NOS1 in the development and progression of different tumors. Indeed, its overexpression is associated with ovarian cell proliferation and invasion as well as with chemoresistance [303,304]. Further studies confirmed the association between NOS1 expression and drug resistance in other tumors such as melanoma where NOS1 overexpression led to a low response to interferon [305,306]. As regards breast cancer, no studies on the effect of neuronal NOS (nNOS/NOS1) are reported in the literature; however, it was demonstrated how NOS1AP is able to bind other proteins, SCRIB and VANGL1, regulating different features of breast cancer cells including cell polarity, migration, and progression suggesting how this protein could be involved in the pathogenesis of this tumor when dysregulated [307]. In addition, other studies on neurodegenerative disorders demonstrated the strict interaction existing between NOS1AP and NOS1 identifying potential targetable binding sites useful to regulate the effect of the interactions of these two proteins [308]. Similarly, also the interactions existing between NOS1 and NOSIP have been investigated; however, the functional effects of these interactions in cancer are not fully understood yet [309,310]. All these data together with the most recent findings on the efficacy of nitric oxide-targeted therapy in estrogen receptor-positive breast cancer cells [280] suggest that the entire NO pathway could play a key role in breast cancer.

Clearly, further studies must be done in order to examine the relationship between the various subsets of breast cancer and iNOS. While it is clear that the overexpression of iNOS is associated with breast cancer and other cancer malignancies, there are also reports that the expression of iNOS/NO is also associated with tumor suppression. Vannini et al. have reviewed the dual role of NO in cancer and reported several cancer models where the expression of NO inhibited tumor growth and metastasis [3]. In addition, the iNOS expression by non-cancer cells in the TME was associated with tumor inhibition and reduced metastases in model systems.

9. Conclusions

Overall, the above findings have indicated that iNOS/NO is involved in the regulation of the protooncogenes, HER2, BRCA1, and BRCA2. NO and RNS also play a role in promoting gene mutations. Therefore, clearly both BRCA1 and BRCA2 are susceptible to mutations and play a large role in the pathogenesis of breast and ovarian cancer. More investigations are warranted to examine the direct relationship between iNOS/NO,
BRCA1/2 mutations, and the onset of tumor development. Likewise, the roles of iNOS/NO in the regulation of HER2 transcription, expression, and onset of tumorigenesis are to be determined. Such investigations may be useful for therapeutic interventions at early stages and also as preventive measures in the future.

**Author Contributions:** Conceptualization, B.B.; methodology (K.L., S.V., L.F., M.L.); validation (L.F., M.L., A.S.); formal analysis (B.B., K.L., S.B., L.F., M.L.); writing original draft preparation 9 K.L., B.B., S.B.); writing-review and editing (K.L., B.B., S.B.); supervision (B.B.). All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** We acknowledge the Department of Microbiology, Immunology & Molecular Genetics and the David Geffen School of Medicine for their assistance and help in the preparation of this manuscript. (B.B.). This work was supported by the Italian League Against Cancer (LILT)-Grant Ricerca Sanitaria 2018 LILT (M.L.; A.S.).

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

- 8-OH-dg: Deoxyguanosine
- A549: Adenocarcinomic human alveolar basal epithelial cells
- ADR: Adriamycin
- Akt: Activated turbidimetric-kinetic
- AP-1: Activating protein-1
- ATF 2: Activating transcription factor 2
- ATM: Ataxia-telangiectasia mutated
- ATR: ATM and Rad-3 related
- β-hCG: Beta human chorionic gonadotropin
- BACH1: BTB domain and CNC homology 1
- BCL11A: B-cell leukemia/lymphoma
- BCLC: Breast Cancer Linkage Consortium
- BRCA1/2: Breast cancer susceptibility gene
- Bp: Base pair
- BRCT: BRCA1 C terminus
- Ca2+: Calcium
- CaM: Calmodulin
- CAT: Chloramphenicol acetyltransferase
- CBP: CREB binding protein
- CCDC98: Coiled-coil domain-containing protein
- CD1: Cluster of differentiation 1
- Cd2 1/4/6: Cyclin-dependent kinase 2/4/6
- CEBPβs: CCAAT-enhancer-binding proteins
- cGMP/PKG: Cyclic guanosine monophosphate/protein kinase G
- ChIP: Chromatin immunoprecipitation
- Chk1: Checkpoint protein 1
- CK1: Casein kinase 1
- C-myc: Cellular myc
- cNOS: Constitutive nitric oxide synthase
- COX-2: Cyclooxygenase 2
- CREB: cAMP-response element binding protein
- CREM: cAMP-responsive element modulator
- CSC: Cancer stem cell
- CtBP1: Carboxyl-terminal binding protein 1
- CtIP: COUP-TF interacting protein
| Abbreviation | Full Form |
|--------------|-----------|
| Cys          | Cysteine  |
| DNA          | Deoxyribonucleic acid |
| E1A          | Adenovirus early region 1A |
| E2/E3        | Estradiol 2/3 |
| E2F1/4       | E2 transcription factor 1/4 |
| EGCG         | Epigallocatechin-3 |
| EGFR         | Epidermal growth factor receptor |
| EMSA         | Electrophoretic mobility shift assay |
| eNOS/NOS3    | Endothelial nitric oxide synthase |
| EOC          | Epithelial ovarian carcinoma |
| ER           | Estrogen receptor-negative breast cancer |
| ERα          | Estrogen receptor |
| ER-β         | Estrogen receptor-β |
| ErbB2        | Erythroblastic oncogene B2 |
| ERK          | Extracellular-signal-regulated kinase |
| ERM          | Ezrin, radixin, and moesin |
| EMT          | Epithelial–mesenchymal transition |
| Ets-1        | Erythroblast transformation specific-1 |
| FISH         | Fluorescence in situ hybridization |
| G2/M         | Growth 2/mitosis |
| GABP-α/β     | GA binding protein |
| GAPDH        | Glyceraldehyde-3-phosphate-dehydrogenase |
| GATA-1       | GATA-binding factor-1/erythroid transcription factor |
| H3K9         | Histone H3 lysine 9 |
| HCC 38       | Hepatocellular carcinoma |
| HDAC         | Histone deacetylases |
| Hdm2         | Human double minute 2 |
| HER2/neu     | Human epidermal growth factor receptor 2 |
| HR           | Homologous recombination |
| HT-29        | Human colon adenocarcinoma cell line |
| ID 1/4       | Inhibitor of differentiation 1/4 |
| IFN-γ        | Interferon gamma |
| IHC          | Immunohistochemistry |
| IL-1/2/4/8   | Interleukin 1/2/4/8 |
| IL-1β        | Interleukin 1 beta |
| iNOS         | Inducible nitric oxide synthase |
| KRAS         | Kirsten rat sarcoma viral oncogene homolog |
| L. donovani   | Leishmania donovani |
| IncRNA       | Long non-coding RNA |
| L-NMMA       | NG-monomethyl-L-arginine monoacetate |
| LOH          | Loss of heterozygosity |
| MAPK         | Mitogen-activated protein kinase |
| MCF-7        | Michigan Cancer Foundation-7 |
| MEK          | Mitogen-activated extracellular signal-regulated kinase |
| miRNA        | MicroRNA |
| MMC          | Mitomycin C |
| MMP 0/2/9    | Matrix metalloproteinase 0/2/9 |
| mRNA         | Messenger RNA |
| ncRNA        | non-coding RNA |
| NF-kB        | Nuclear Factor kappa-light-chain-enhancer of activated B cells |
| NPAT-5/C4    | Nuclear factor of activated T cells 5/C4 |
| NHEJ         | Nonhomologous end-joining |
| NLS          | Nuclear localization sequences |
| nNOS/NOS1    | Neuronal nitric oxide synthase |
| NO           | Nitric oxide |
NOSIP  Nitric oxide synthase interacting protein
NOS1AP  Nitric oxide synthase 1 adaptor protein
NOTCH-1  NOTCH homolog 1
Oct  Octamer factor
PANC-1  Human pancreatic cancer cell line
p53  Tumor suppressor p53
PALB2  Partner and localizer of BRCA2
PARP  Poly(ADP-ribose) polymerase
PCAT-1  Prostate cancer associated transcript 1
PCR  Polymerase chain reaction
PDAC  Pancreatic adenocarcinoma
PGE2  Prostaglandin E2
PI3K  Phosphoinositide 3 kinase
qPCR  Quantitative polymerase chain reaction
Ras  Rat Sarcoma Virus
RB  Retinoblastoma protein
Rb-E2F  Retinoblastoma protein family-activator E2F
RBL2  RB Transcriptional corepressor like 2
RING  Really interesting new gene
RKO  Rectal carcinoma cell line
RNA  Ribonucleic acid
RNAseq  RNA sequence
RNS  Reactive nitrogen species
RR  Ribonucleotide reductase
RT-PCR  Reverse transcription PCR
RUNX1  Runt-related transcription factor 1
S phase  Synthesis phase
S100B  S100 calcium-binding protein B
shRNA  Short hairpin RNA
siRNA  Small interfering RNA
Slug  Snail 1/2
Sp-1  Specificity protein 1
SNAP  S-nitroso-N-acetylpenicillamine
STAT-1α  Signal transducer and activator of transcription 1
SV40  Simian vacuolating virus 40
TCGA  The Cancer Genome Atlas
TGFBR II  Transforming growth factor beta receptor type II gene
TME  Tumor microenvironment
TNBC  Triple-negative breast cancer
TNF  Tumor necrosis factor
TNF-α  Tumor necrosis factor alpha
Trp53  Transformation related protein 53
TUNEL  Terminal deoxynucleotidyl transferase dUTP nick end labeling
USF  Upstream stimulatory factor
UTR  Untranslated region
VEGF  Vascular endothelial growth factor

References
1. Garcea, G.; Dennison, A.R.; Steward, W.P.; Berry, D.P. Role of Inflammation in Pancreatic Carcinogenesis and the Implications for Future Therapy. *Pancreatology* 2005, 5, 514–529. [CrossRef] [PubMed]
2. Pervin, S.; Singh, R.; Sen, S.; Chaudhuri, G. Dual Role of Nitric Oxide in Cancer Biology. In *Nitric Oxide (NO) and Cancer: Prognosis, Prevention, and Therapy*; Bonavida, B., Ed.; Cancer Drug Discovery and Development; Springer: New York, NY, USA, 2010; pp. 39–57. [CrossRef]
3. Vannini, F.; Kashfi, K.; Nath, N. The Dual Role of INOS in Ovarian Cancer. *Redox Biol.* 2015, 6, 334–343. [CrossRef] [PubMed]
4. Kielbik, M.; Szulc-Kielbik, I.; Klink, M. The Potential Role of INOS in Ovarian Cancer Progression and Chemoresistance. *Int. J. Mol. Sci.* 2019, 20, 1751. [CrossRef] [PubMed]
5. Kashfi, K.; Kannikal, J.; Nath, N. Macrophage Reprogramming and Cancer Therapeutics: Role of INOS-Derived NO. *Cells* 2021, 10, 3194. [CrossRef] [PubMed]
6. Vivarella, S.; Falzone, L.; Basile, M.S.; Candido, S.; Libra, M. Nitric Oxide in Hematological Cancers: Partner or Rival? *Antioxid. Redox Signal*. 2021, 34, 383–401. [CrossRef] [PubMed]

7. Zhang, R.; Ma, A.; Urbanski, S.J.; McCallery, D.-K. Induction of Inducible Nitric Oxide Synthase: A Protective Mechanism in Collitis-Induced Adenocarcinoma. *Carcinogenesis* 2007, 28, 1122–1130. [CrossRef]

8. Wink, D.A.; Ridnour, L.A.; Hussain, S.P.; Harris, C.C. The Reemergence of Nitric Oxide and Cancer. *Nitric Oxide* 2008, 19, 65–67. [CrossRef]

9. Xie, K.; Huang, S.; Dong, Z.; Juang, S.H.; Gutman, M.; Xie, Q.W.; Nathan, C.; Fidler, I.J. Transfection with the Inducible Nitric Oxide Synthase Gene Suppresses Tumorigenicity and Abrogates Metastasis by K-1753 Murine Melanoma Cells. *J. Exp. Med.* 1995, 181, 1333–1343. [CrossRef]

10. Xu, W.; Liu, L.Z.; Loizidou, M.; Ahmed, M.; Charles, I.G. The Role of Nitric Oxide in Cancer. *Cell Res.* 2002, 12, 311–320. [CrossRef]

11. Burke, A.J.; Sullivan, F.J.; Giles, F.J.; Glynn, S.A. The Yin and Yang of Nitric Oxide in Cancer Progression. *Carcinogenesis* 2013, 34, 503–512. [CrossRef]

12. Vane, J.R.; Mitchell, J.A.; Appleton, I.; Tomlinson, A.; Bishop-Bailey, D.; Croxtall, J.; Willoughby, D.A. Inducible Isoforms of Cyclooxygenase and Nitric-Oxide Synthase in Inflammation. *Proc. Natl. Acad. Sci. USA* 1994, 91, 2046–2050. [CrossRef] [PubMed]

13. Yakovlev, V.A. Nitric Oxide–Dependent Downregulation of BRCA1 Expression Promotes Genetic Instability. *Cancer Res.* 2013, 73, 706–715. [CrossRef] [PubMed]

14. Garrido, P.; Shalaby, A.; Walsh, E.M.; Keane, N.; Webber, M.; Keane, M.M.; Sullivan, F.J.; Kerin, M.J.; Callagy, G.; Ryan, A.E.; et al. Impact of Inducible Nitric Oxide Synthase (iNOS) Expression on Triple Negative Breast Cancer Outcome and Activation of EGFR and ERK Signaling Pathways. *Oncotarget* 2017, 8, 80568–80588. [CrossRef]

15. Chen, D.-M.; Shi, Z.-Q.; Tan, L.-L.; Chen, Y.-P.; Li, C.-Q.; Wang, Q.; Li, H.; Zhang, M.-L.; Song, J.-P.; Xu, Q.; et al. Short-Hairpin RNA-Guided Single Gene Knockdown Reverses Triple-Negative Breast Cancer. *bioRxiv* 2018, 418764. [CrossRef]

16. Mishra, D.; Patel, V.; Banerjee, D. Nitric Oxide and S-Nitrosylation in Cancers: Emphasis on Breast Cancer. *Breast Cancer* 2020, 14, 1178223419882688. [CrossRef] [PubMed]

17. Thomsen, L.L.; Miles, D.W.; Happerfield, L.; Bobrow, L.G.; Knowles, R.G.; Moncada, S. Nitric Oxide Synthase Activity in Human Breast Cancer. *Br. J. Cancer* 1995, 72, 41–44. [CrossRef]

18. Thomsen, L.L.; Lawton, F.G.; Knowles, R.G.; Beesley, J.E.; Riveros-Moreno, V.; Moncada, S. Nitric Oxide Synthase Activity in Human Gynecological Cancer. *Cancer Res.* 1994, 54, 1352–1354. [CrossRef]

19. Kamfaran, G.; D ores, G.M.; Anderson, W.F. Patterns of Cancer Incidence, Mortality, and Prevalence across Five Continents: Defining Priorities to Reduce Cancer Disparities in Different Geographic Regions of the World. *J. Clin. Oncol.* 2006, 24, 2137–2150. [CrossRef]

20. Polyak, K. Breast Cancer: Origins and Evolution. *J. Clin. Investig.* 2007, 117, 3155–3163. [CrossRef]

21. Perou, C.M.; Sorlie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; Rees, C.A.; Pollack, J.R.; Ross, D.T.; Johnsen, H.; Akslen, L.A.; et al. Molecular Portraits of Human Breast Tumours. *Nature* 2000, 406, 747–752. [CrossRef]

22. Sørlie, T.; Perou, C.M.; Tibshirani, R.; Aas, T.; Geisler, S.; Johnsen, S.; Hastie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; et al. Gene Expression Patterns of Breast Carcinomas Distinguish Tumor Subclasses with Clinical Implications. *Proc. Natl. Acad. Sci. USA* 2001, 98, 10869–10874. [CrossRef] [PubMed]

23. Osborne, C.; Wilson, P.; Tripathy, D. Oncogenes and Tumor Suppressor Genes in Breast Cancer: Potential Diagnostic and Therapeutic Applications. *Oncologist* 2004, 9, 361–377. [CrossRef] [PubMed]

24. Torry, D.S.; Cooper, G.M. Proto-Oncogenes in Development and Cancer. *Am. J. Reprod. Immunol.* 1993, 25, 129–132. [CrossRef] [PubMed]

25. Yarden, Y.; Slwikowski, M.X. Untangling the ErbB Signalling Network. *Nat. Rev. Mol. Cell Biol.* 2001, 2, 127–137. [CrossRef] [PubMed]

26. Satyanarayanananjos, S.; Villalba, S.; Jianchao, L.; Lin, G.M. Design, Synthesis, and Docking Studies of Peptidomimetics Based on HER2–Herceptin Binding Site with Potential Antiproliferative Activity against Breast Cancer Cell Lines. *Chem. Biol. Drug Des.* 2009, 74, 246–257. [CrossRef] [PubMed]

27. Huang, H.-J.; Lee, K.-J.; Yu, H.W.; Hsu, C.-H.; Chen, H.-Y.; Tsai, F.-J.; Chen, C.Y.-C. Structure-Based and Ligand-Based Drug Design for HER 2 Receptor. *J. Biomol. Struct. Dyn.* 2010, 28, 23–37. [CrossRef] [PubMed]

28. Slamon, D.J.; Clark, G.M.; Wong, S.G.; Levin, W.J.; Ullrich, A.; McGuire, W.L. Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/Neu Oncogene. *Science* 1987, 235, 177–182. [CrossRef]

29. Slamon, D.J.; Godolphin, W.; Jones, L.A.; Holt, J.A.; Wong, S.G.; Keith, D.E.; Levin, W.J.; Stuart, S.G.; Udove, J.; Ullrich, A.; et al. Studies of the HER-2/Neu Proto-Oncogene in Human Breast and Ovarian Cancer. *Science* 1989, 244, 707–712. [CrossRef]

30. Tandon, A.K.; Clark, G.M.; Chamness, G.C.; Ullrich, A.; McGuire, W.L. HER-2/Neu Oncogene Protein and Prognosis in Breast Cancer. *J. Clin. Oncol.* 1999, 17, 1120–1128. [CrossRef]

31. Seshadri, S.; Firgaira, F.A.; Horsfall, D.J.; McCaul, K.; Setlur, V.; Kitchen, P. Clinical Significance of HER-2/Neu Gene Expression but Not with Ras Gene Mutations. *J. Natl. Cancer Inst.* 1993, 85, 897–901. [CrossRef] [PubMed]
33. Ding, Q.; Huo, L.; Yang, J.-Y.; Xia, W.; Wei, Y.; Liao, Y.; Chang, C.-J.; Yang, Y.; Lai, C.-C.; Lee, D.-F.; et al. Down-Regulation of Myeloid Cell Leukemia-1 through Inhibiting Erk/PI3 Pathway by Sorafenib Facilitates Chemosensitization in Breast Cancer. Cancer Res. 2008, 68, 6109–6117. [CrossRef] [PubMed]

34. Sheu, J.-J.-C.; Hsu, C.-H.; Wan, L.; Lin, Y.-J.; Lai, M.-T.; Tseng, H.-C.; Jinawath, N.; Tsai, M.-H.; Chang, N.-W.; Lin, C.-F.; et al. Functional Genomic Analysis Identified Epidermal Growth Factor Receptor Activation as the Most Common Genetic Event in Oral Squamous Cell Carcinoma. Cancer Res. 2009, 69, 2559–2576. [CrossRef]

35. van de Vijver, M.J.; Petersen, J.L.; Mooi, W.J.; Lomans, J.; Dalesio, O.; Nusse, R. Neu-Protein Overexpression in Breast Cancer. N. Engl. J. Med. 1988, 319, 1239–1245. [CrossRef] [PubMed]

36. Miller, S.; Hung, M. Regulation of HER-2/neu gene expression (Review). Oncol. Rep. 1995, 2, 497–503. [CrossRef]

37. Chen, H.; Hung, M.-C. Involvement of Co-Activator P300 in the Transcriptional Regulation of the HER-2/Neu Gene. Oncol. Rep. 1997, 272, 6101–6104. [CrossRef]

38. Yang, G.; Cai, K.Q.; Thompson-Lanza, J.A.; Bast, R.C.; Liu, J. Inhibition of Breast and Ovarian Tumor Growth through Multiple Signaling Pathways by Using Retrovirus-Mediated Small Interfering RNA against HER-2/Neu Gene Expression. J. Biol. Chem. 2004, 279, 4339–4345. [CrossRef]

39. Elbashir, S.M.; Harborth, J.; Lendeckel, W.; Yalcin, A.; Weber, K.; Tuschl, T. Duplexes of 21-Nucleotide RNAs Mediate RNA Interference in Cultured Mammalian Cells. Nature 2001, 411, 494–498. [CrossRef]
60. Choudhury, A.; Charo, J.; Parapuram, S.K.; Hunt, R.C.; Hunt, D.M.; Seliger, B.; Kiessling, R. Small Interfering RNA (SiRNA) Inhibits the Expression of the Her2/Neu Gene, Upregulates HLA Class I and Induces Apoptosis of Her2/Neu Positive Tumor Cell Lines. *Int. J. Cancer* 2004, 108, 71–77. [CrossRef]

61. Sengodan, S.K.; Sreelatha, K.H.; Nadhan, R.; Srinivas, P. Regulation of Epithelial to Mesenchymal Transition by BRCA1 in Breast Cancer. *Crit. Rev. Oncol./Hematol.* 2018, 123, 74–82. [CrossRef]

62. Diaz-Cruz, E.S.; Cabrera, M.C.; Nakles, R.; Rutstein, B.H.; Furth, P.A. BRCA1 Deficient Mouse Models to Study Pathogenesis and Therapy of Triple Negative Breast Cancer. *Breast Dis.* 2011, 32, 85–97. [CrossRef] [PubMed]

63. de Ruiter, T.C.; Veek, J.; de Hoon, J.P.J.; van Engelund, M.; Tjän-Heijnen, V.C. Characteristics of Triple-Negative Breast Cancer. *J. Cancer Res. Clin. Oncol.* 2011, 137, 183–192. [CrossRef] [PubMed]

64. Deng, C.-X. BRCA1: Cell Cycle Checkpoint, Genetic Instability, DNA Damage Response and Cancer Evolution. *Nucleic Acids Res.* 2006, 34, 1416–1426. [CrossRef] [PubMed]

65. Mullan, P.B.; Quinn, J.E.; Harkin, D.P. The Role of BRCA1 in Transcriptional Regulation and Cell Cycle Control. *Oncogene* 2006, 25, 5854–5863. [CrossRef]

66. Diaz-Cruz, E.S.; Cabrera, M.C.; Nakles, R.; Rutstein, B.H.; Furth, P.A. BRCA1 Deficient Mouse Models to Study Pathogenesis and Therapy of Triple Negative Breast Cancer. *Breast Dis.* 2011, 32, 85–97. [CrossRef] [PubMed]

67. Hosseyni, A.M.; Gorski, J.J.; Murray, M.M.; Quinn, J.E.; Chung, W.Y.; Stewart, G.E.; James, C.R.; Farragher, S.M.; Mulligan, J.M.; Scott, A.N.; et al. Molecular Basis for Estrogen Receptor α Deficiency in BRCA1-Linked Breast Cancer. *J. Natl. Cancer Inst.* 2007, 99, 1683–1694. [CrossRef]

68. Mueller, C.R.; Roskelley, C.D. Regulation of BRCA1 Expression and Its Relationship to Sporadic Breast Cancer. *Breast Cancer Res.* 2002, 5, 45. [CrossRef]

69. Ye, Q.; Hu, Y.-F.; Zhong, H.; Nye, A.C.; Belmont, A.S.; Li, R. BRCA1-Induced Large-Scale Chromatin Unfolding and Allele-Specific Effects of Cancer-Predisposing Mutations. *J. Cell Biol.* 2001, 155, 911–922. [CrossRef]

70. Hosey, A.M.; Gorski, J.J.; Murray, M.M.; Quinn, J.E.; Chung, W.Y.; Stewart, G.E.; James, C.R.; Farragher, S.M.; Mulligan, J.M.; Scott, A.N.; et al. Molecular Basis for Estrogen Receptor α Deficiency in BRCA1-Linked Breast Cancer. *J. Natl. Cancer Inst.* 2007, 99, 1683–1694. [CrossRef]

71. Yarden, R.I.; Pardo-Reoyo, S.; Sgagias, M.; Cowan, K.H.; Brody, L.C. BRCA1 Regulates the G2/M Checkpoint by Activating Chk1 Kinase upon DNA Damage. *Nat. Genet.* 2002, 30, 285–289. [CrossRef]

72. Claus, J.; Valderrama, F. ERM Proteins in Cancer Progression. *J. Cell Sci.* 2015, 128, 1253. [CrossRef]

73. Coene, E.D.; Gadelha, C.; White, N.; Malhas, A.; Thomas, B.; Shaw, M.; Vaux, D.J. A Novel Role for BRCA1 in Regulating Breast Cancer Cell Spreading and Motility. *J. Cell Biol.* 2011, 192, 497–512. [CrossRef] [PubMed]

74. Ye, Q.; Hu, Y.-F.; Zhong, H.; Nye, A.C.; Belmont, A.S.; Li, R. BRCA1-Induced Large-Scale Chromatin Unfolding and Allele-Specific Effects of Cancer-Predisposing Mutations. *J. Cell Biol.* 2001, 155, 911–922. [CrossRef]

75. Lindeman, G.J.; Visvader, J.E. Cell Fate Takes a Slug in BRCA1-Associated Breast Cancer. *Breast Cancer Res.* 2011, 13, 306. [CrossRef]

76. Paull, T.T.; Cortez, D.; Bowers, B.; Elledge, S.J.; Gellert, M. Direct DNA Binding by Brca1. *Proc. Natl. Acad. Sci. USA* 2001, 98, 6086–6091. [CrossRef]

77. Xu, X.; Weaver, Z.; Linke, S.P.; Li, C.; Gotay, J.; Wang, X.-W.; Harris, C.C.; Ried, T.; Deng, C.-X. Centrosome Amplification and a Centrosome Checkpoint Induce Genetic Instability in BRCA1 Exon 11 Isoform–Deficient Cells. *Proc. Natl. Acad. Sci. USA* 2001, 98, 6086–6091. [CrossRef]

78. Irwin, J.J. Community Benchmarks for Virtual Screening. *J. Comput. Aided Mol. Des.* 2008, 22, 193–199. [CrossRef]

79. Clark, S.L.; Rodriguez, A.M.; Snyder, R.R.; Hankins, G.D.V.; Boehning, D. Structure-function of the tumor suppressor BRCA1. *Comput. Struct. Biotechnol. J.* 2015, 2, e2014005. [CrossRef]

80. Greenberg, R.A. BRCA1, Everything but the RING? *Science* 2001, 293, 459–460. [CrossRef]

81. Shakya, R.; Reid, L.J.; Rezcek, C.R.; Cole, F.; Eglie, D.; Lin, C.-S.; deRooij, D.G.; Ravi, K.; Hicks, J.B.; et al. BRCA1 Tumor Suppression Depends on BRCT Phosphoprotein Binding, But Not Its E3 Ligase Activity. *Proc. Natl. Acad. Sci. USA* 2001, 98, 6086–6091. [CrossRef]

82. Ye, Q.; Hu, Y.-F.; Zhong, H.; Nye, A.C.; Belmont, A.S.; Li, R. BRCA1-Induced Large-Scale Chromatin Unfolding and Allele-Specific Effects of Cancer-Predisposing Mutations. *J. Cell Biol.* 2001, 155, 911–922. [CrossRef]

83. Mohammad, D.H.; Yaffe, M.B. 14-3-3 Proteins, FHA Domains and BRCT Domains in the DNA Damage Response. *Cell Cycle* 2008, 7, 3781–3783. [CrossRef] [PubMed]

84. Proia, T.A.; Keller, P.J.; Gupta, P.B.; Klebba, I.; Jones, A.D.; Sedic, M.; Gellert, M. Direct DNA Binding by BRCA1. *Proc. Natl. Acad. Sci. USA* 2001, 98, 6086–6091. [CrossRef]

85. Hosey, A.M.; Gorski, J.J.; Murray, M.M.; Quinn, J.E.; Chung, W.Y.; Stewart, G.E.; James, C.R.; Farragher, S.M.; Mulligan, J.M.; Scott, A.N.; et al. Molecular Basis for Estrogen Receptor α Deficiency in BRCA1-Linked Breast Cancer. *J. Natl. Cancer Inst.* 2007, 99, 1683–1694. [CrossRef]

86. Proia, T.A.; Keller, P.J.; Gupta, P.B.; Klebba, I.; Jones, A.D.; Sedic, M.; Gellert, M. Direct DNA Binding by BRCA1. *Proc. Natl. Acad. Sci. USA* 2001, 98, 6086–6091. [CrossRef]

87. Lane, T.F.; Deng, C.; Elson, A.; Lyu, M.S.; Kozak, C.A.; Leder, P. Expression of Brca1 Is Associated with Terminal Differentiation of Ectodermally and Mesodermally Derived Tissues in Mice. *Genes Dev.* 1995, 9, 2712–2722. [CrossRef]

88. Marquis, S.T.; Rajan, J.V.; Wynshaw-Boris, A.; Xu, J.; Yin, G.-Y.; Abel, K.J.; Weber, B.L.; Chodosh, L.A. The Developmental Pattern of Brca1 Expression Implies a Role in Differentiation of the Breast and Other Tissues. *Nat. Genet.* 1995, 11, 17–26. [CrossRef]
90. Jeffy, B.D.; Hockings, J.K.; Kemp, M.Q.; Morgan, S.S.; Hager, J.A.; Belyakoff, J.; Whitesell, L.J.; Bowden, G.T.; Romagnolo, D.F. An Estrogen Receptor-α/P300 Complex Promotes BRCA1 Inhibitor at an AI-1 Site That Binds Jun/Fos Transcription Factors: Repressive Effects of P53 on BRCA1 Transcription. *Neoplasia* 2005, 7, 873–882. [CrossRef]

91. Fan, S.; Ma, Y.X.; Wang, C.; Yuan, R-Q.; Meng, Q.; Wang, J-A.; Erdos, M.; Goldberg, I.D.; Webb, P.; Kushner, P.J.; et al. P300 Modulates the BRCA1 Inhibition of Estrogen Receptor Activity. *Cancer Res.* 2002, 62, 141–151.

92. Hall, J.M.; Lee, M.K.; Newman, B.; Morrow, J.E.; Anderson, L.A.; Huey, B.; King, M.-C. Linkage of Early-Onset Familial Breast Cancer to Chromosome 17q21. *Science* 1990, 250, 1684–1689. [CrossRef] [PubMed]

93. Ford, D.; Easton, D.F.; Stratton, M.; Narod, S.; Goldgar, D.; Devilee, P.; Bishop, D.T.; Weber, B.; Lenoir, G.; Chang-Claude, J.; et al. Genetic Heterogeneity and Penetration Analysis of the BRCA1 and BRCA2 Genes in Breast Cancer Families. *Am. J. Hum. Genet.* 1998, 62, 676–689. [CrossRef] [PubMed]

94. Easton, D.F.; Bishop, D.T.; Ford, D.; Crockford, G.P. Genetic Linkage Analysis in Familial Breast and Ovarian Cancer: Results from 214 Families. The Breast Cancer Linkage Consortium. *Am. J. Hum. Genet.* 1993, 52, 678–701. [PubMed]

95. Narod, S.A.; Ford, D.; Devilee, P.; Barkdottir, R.B.; Lynch, H.T.; Smith, S.A.; Ponder, B.A.J.; Weber, B.L.; Garber, J.E.; Birch, J.M.; et al. An Evaluation of Genetic Heterogeneity in 145 Breast-Ovarian Cancer Families. *Am. J. Hum. Genet.* 1995, 56, 254–264.

96. Wooster, R.; Neuhausen, S.L.; Mangion, J.; Quirk, Y.; Ford, D.; Collins, N.; Nguyen, K.; Seal, S.; Tran, T.; Averill, D.; et al. Localization of a Breast Cancer Susceptibility Gene, BRCA2, to Chromosome 13q12-13. *Science* 1994, 265, 2088–2090. [CrossRef]

97. Kinzler, K.W.; Vogelstein, B. Gatekeepers and Caretakers. *Nature* 1997, 386, 761–763. [CrossRef]

98. Crook, T.; Crossland, S.; Crompton, M.R.; Osin, P.; Gusterson, B.A. P53 Mutations in BRCA1-Associated Familial Breast Cancer. *Lancet* 1997, 350, 638–639. [CrossRef]

99. Crook, T.; Brooks, L.A.; Crossland, S.; Osin, P.; Barker, K.T.; Waller, J.; Philp, E.; Smith, P.D.; Yulug, I.; Peto, J.; et al. P53 Mutation with Frequent Novel Codons but Not a Mutator Phenotype in BRCA1 and BRCA2-Associated Breast Tumours. *Oncogene* 1998, 17, 1681–1689. [CrossRef]

100. Palacios, J.; Honrado, E.; Osorio, A.; Cazorla, A.; Sarrió, D.; Barroso, A.; Rodriguez, S.; Cigudosa, J.C.; Diez, O.; Alonso, C.; et al. Phenotypic Characterization of BRCA1 and BRCA2 Tumors Based in a Tissue Microarray Study with 37 Immunohistochemical Markers. *Breast Cancer Res. Treat.* 2003, 90, 5–14. [CrossRef]

101. Aaltonen, K.; Blomqvist, C.; Amini, R.-M.; Eerola, H.; Aittomäki, K.; Heikkilä, P.; Nevanlinna, H. Familial Breast Cancers without Mutations in BRCA1 or BRCA2 Have Low Cylcin E and High Cylcin D1 in Contrast to Cancers in BRCA Mutation Carriers. *Clin. Cancer Res.* 2008, 14, 1976–1983. [CrossRef]

102. Sergoian, S.K.; Nadhan, R.; Nair, R.S.; Hemalatha, S.K.; Somasundaram, V.; Sushama, R.R.; Rajan, A.; Latha, N.R.; Varghese, G.R.; Thankappan, R.K.; et al. BRCA1 Regulation on β-HCG: A Mechanism for Tumorigenicity in BRCA1 Defective Breast Cancer. *Oncogenesis* 2017, 6, e376. [CrossRef] [PubMed]

103. Stenman, U.-H.; Alfthan, H.; Hotakainen, K. Human Chorionic Gonadotropin in Cancer. *Clin. Biochem.* 2004, 37, 549–561. [CrossRef] [PubMed]

104. Tonio, P.; Grankvist, K.; Wulff, M.; Chen, T.; Johansson, R.; Schock, H.; Lenner, P.; Hallmans, G.; Lehtinen, M.; Kaaks, E.; et al. Human Chorionic Gonadotropin in Pregnancy and Maternal Risk of Breast Cancer. *Cancer Res.* 2010, 70, 6779–6786. [CrossRef]

105. Gillott, D.J.; Iles, R.K.; Chard, T. The Effects of Beta-Human Chorionic Gonadotrophin on the in Vitro Growth of Bladder Cancer Neoplasia. *2005, 7, 873–882. [CrossRef]

106. Jankowska, A.; Gunderson, S.I.; Andrusiewicz, M.; Burczynska, B.; Szczerba, A.; Jarmolowski, A.; Nowak-Markwitz, E.; Warchol, J.B. Reduction of Human Chorionic Gonadotropin Beta Subunit Expression by Modified U1 SnRNA Caused Apoptosis in Cervical Human Epithelial Cells. *Am. J. Pathol.* 2000, 156, 1385–1393. [CrossRef] [PubMed]

107. Guo, X.; Liu, G.; Schneider-Broussard, R.; Kumar, A.P.; MacLeod, M.C.; Johnson, D.G. Regulation of BRCA1 Expression by the Rb-E2F Pathway. *J. Biol. Chem.* 2005, 280, 26463–26470. [CrossRef] [PubMed]

108. Wan, H.; Versnel, M.A.; Leijten, L.M.E.; van Helden-Meeuwsen, C.G.; Fekkes, D.; Leenen, P.J.M.; Khan, N.A.; Benner, R.; Kiekkens, R.C.M. Chorionic Gonadotropin Induces Dendritic Cells to Express a Tolerogenic Phenotype. *J. Leukoc. Biol.* 2008, 83, 894–901. [CrossRef]

109. Tanizaki, J.; Ercan, D.; Capelletti, M.; Dodge, M.; Xu, C.; Bahcall, M.; Tricker, E.M.; Butaney, M.; Alles, I.A.; Sholl, L.M.; et al. Identification of Oncogenic and Drug-Sensitizing Mutations in the Extracellular Domain of FGFR2. *Cancer Res.* 2015, 75, 3139–3146. [CrossRef] [PubMed]

110. Deng, Y.; Deng, H.; Liu, J.; Han, G.; Malkoski, S.; Liu, B.; Zhao, R.; Wang, X.-J.; Zhang, Q. Transcriptional Down-Regulation of Brca1 and E-Cadherin by CtBP1 in Breast Cancer. *Mol. Carcinog.* 2012, 51, 500–507. [CrossRef]

111. Wang, A.; Schneider-Broussard, R.; Kumar, A.P.; MacLeod, M.C.; Johnson, D.G. Regulation of BRCA1 Expression by the Rb-E2F Pathway. *J. Biol. Chem.* 2000, 275, 4532–4536. [CrossRef]

112. Tao, Y.; Kassatly, R.F.; Cress, W.D.; Horowitz, J.M. Subunit Composition Determines E2F DNA-Binding Site Specificity. *Mol. Cell. Biol.* 1997, 17, 6994–7007. [CrossRef] [PubMed]

113. Almasan, A.; Yin, Y.; Kelly, R.E.; Lee, E.Y.; Bradley, A.; Li, W.; Bertino, J.R.; Wahl, G.M. Deficiency of Retinoblastoma Protein Leads to Inappropriate S-Phase Entry, Activation of E2F2 Genes, and Apoptosis. *Proc. Natl. Acad. Sci. USA* 1995, 92, 5436–5440. [CrossRef] [PubMed]
114. Herrera, R.E.; Sah, V.P.; Williams, B.O.; Mäkelä, T.P.; Weinberg, R.A.; Jacks, T. Altered Cell Cycle Kinetics, Gene Expression, and G1 Restriction Point Regulation in Rh-Deficient Fibroblasts. *Mol. Cell. Biol.* 1996, 16, 2402–2407. [CrossRef] [PubMed]

115. Hurford, R.K.; Cobrinik, D.; Lee, M.H.; Dyson, N. PRB and P107/P130 Are Required for the Regulated Expression of Different Sets of E2F Responsive Genes. *Genes Dev.* 1997, 11, 1447–1463. [CrossRef]

116. Rice, J.C.; Massey-Brown, K.S.; Futscher, B.W. Aberrant Methylation of the BRCA1 CpG Island Promoter Is Associated with Decreased BRCA1 MRNA in Sporadic Breast Cancer Cells. *Oncogene* 1998, 17, 1807–1812. [CrossRef]

117. Esteller, M.; Corn, P.G.; Baylin, S.B.; Herman, J.G. A Gene Hypermethylation Profile of Human Cancer. *Cancer Res.* 2001, 61, 3225–3229. [PubMed]

118. Jones, P.A.; Laird, P.W. Cancer-Epigenetics Comes of Age. *Nat. Genet.* 1999, 21, 163–167. [CrossRef]

119. Baylin, S.B.; Herman, J.G. DNA Hypermethylation in Tumorigenesis: Epigenetics Joins Genetics. *Trends Genet.* 2000, 16, 168–174. [CrossRef]

120. Lu, Y.; Chu, A.; Turker, M.S.; Glazer, P.M. Hypoxia-Induced Epigenetic Regulation and Silencing of the BRCA1 Promoter. *Mol. Cell. Biol.* 2011, 31, 3339–3350. [CrossRef]

121. Bosviel, R.; Durif, J.; Déchelette, P.; Bignon, Y.-J.; Bernard-Gallon, D. Epigenetic Modulation of BRCA1 and BRCA2 Gene Expression by Equol in Breast Cancer Cell Lines. *Br. J. Nutr.* 2012, 108, 1187–1193. [CrossRef]

122. Garcia, A.I.; Buisson, M.; Bertrand, P.; Rimokh, R.; Rouleau, E.; Lopez, B.S.; Lidereau, R.; Mikaël, I.; Mazoyer, S. Down-regulation of BRCA1 Expression by MiR-146a and MiR-146b-5p in Triple Negative Sporadic Breast Cancers. *EMBO Mol. Med.* 2011, 3, 279–290. [CrossRef] [PubMed]

123. Finnerty, J.R.; Wang, W.-X.; Hébert, S.S.; Wilfred, B.R.; Mao, G.; Nelson, P.T. The MiR-15/107 Group of MicroRNA Genes: Evolutionary Biology, Cellular Functions, and Roles in Human Diseases. *J. Mol. Biol.* 2010, 402, 491–509. [CrossRef] [PubMed]

124. Quann, K.; Jing, Y.; Rigoutsos, I. Post-Transcriptional Regulation of BRCA1 through Its Coding Sequence by the MiR-15/107 Group of MiRNAs. *Front. Genet.* 2015, 6, 242. [CrossRef] [PubMed]

125. Landgraf, P.; Rusu, M.; Sheridan, R.; Sewer, A.; Iovino, N.; Aravin, A.; Pfeffer, S.; Rice, A.; Kamphorst, A.O.; Landthaler, M.; et al. A Mammalian MicroRNA Expression Atlas Based on Small RNA Library Sequencing. *Cell 2007*, 129, 1401–1414. [CrossRef] [PubMed]

126. He, L.; Hannon, G.J. MicroRNAs: Small RNAs with a Big Role in Gene Regulation. *Nat. Rev. Genet.* 2008, 9, 522–531. [CrossRef]

127. Filipowicz, W.; Bhattacharyya, S.N.; Sonenberg, N. Mechanisms of Post-Transcriptional Regulation by MicroRNAs: Are the Answers in Sight? *Nat. Rev. Genet.* 2009, 10, 102–114. [CrossRef]

128. Tavtigian, S.V.; Simard, J.; Rommens, J.; Couch, F.; Shattuck-Eidens, D.; Neuhausen, S.; Merajver, S.; Thorlacius, S.; Offit, K.; Stoppa-Lyonnet, D.; et al. The Complete BRCA2 Gene and Mutations in Chromosome 13q-Linked Kindreds. *Nat. Genet.* 1996, 12, 333–337. [CrossRef]

129. Bertwistle, D.; Swift, S.; Marston, N.J.; Jackson, L.E.; Crossland, S.; Crompton, M.R.; Marshall, C.J.; Ashworth, A. Nuclear Location and Cell Cycle Regulation of the BRCA2 Protein. *Cancer Res.* 1997, 57, 5485–5488. [PubMed]

130. Milner, J.; Ponder, B.; Hughes-Davies, L.; Seltmann, M.; Kouzarides, T. Transcriptional Activation Functions in BRCA2. *Nature* 1997, 386, 772–773. [CrossRef]

131. Chapman, M.S.; Verma, I.M. Transcriptional Activation by BRCA1. *Nature* 1996, 382, 678–679. [CrossRef]

132. Sharan, S.K.; Morimatsu, M.; Albrecht, U.; Lim, D.-S.; Regel, E.; Dinh, C.; Sands, A.; Eichele, G.; Hasty, P.; Bradley, A. Embryonic Lethality and Radiation Hypersensitivity Mediated by Rad51 in Mice Lacking Brca2. *Nature* 1997, 386, 804–810. [CrossRef] [PubMed]

133. Chen, Y.; Chen, P.-L.; Riley, D.J.; Lee, W.-H.; Allred, D.C.; Osborne, C.K. Response: Location of BRCA1 in Human Breast and Ovarian Cancer Cells. *Science* 1996, 272, 125–126. [CrossRef] [PubMed]

134. Bieche, I.; Noguès, C.; Lidereau, R. Overexpression of BRCA2 Gene in Sporadic Breast Tumours. *Oncogene* 1999, 18, 5232–5238. [CrossRef] [PubMed]

135. Callens, N.; Dumont, M.; Begue, A.; Lint, C.; Baert, J.-L.; Simard, J.; Launoit, Y. Genomic Organization and Expression of the Mouse Brca2 Gene. *Mamm. Genome* 2002, 13, 352–358. [CrossRef] [PubMed]

136. Callens, N.; Baert, J.-L.; Monté, D.; Sunes, M.; Van Lint, C.; de Launoit, Y. Transcriptional Regulation of the Murine Brca2 Gene by CREB/ATF Transcription Factors. *Biochem. Biophys. Res. Commun.* 2003, 312, 702–707. [CrossRef] [PubMed]

137. Wu, K.; Jiang, S.-W.; Couch, F.J. P53 Mediates Repression of the BRCA2 Promoter and Down-Regulation of BRCA2 MRNA and Protein Levels in Response to DNA Damage. *J. Biol. Chem.* 2003, 278, 15652–15660. [CrossRef]

138. Lobanova, O.E.; Rossokha, Z.I.; Medvedieva, N.L.; Cheshuk, V.E.; Vershchako, R.I.; Vershyhora, V.O.; Fisshuk, L.Y.; Zakhartseva, L.M.; Gorovenko, N.G. Prevalence of BRCA1 and BRCA2 Genes Promoter Hypermethylation in Breast Cancer Tissue. *Exp. Oncol.* 2021, 43, 56–60. [CrossRef]

139. Dworkin, A.M.; Spearman, A.D.; Tseng, S.Y.; Sweet, K.; Tolan, A.E. Methylation Not a Frequent “Second Hit” in Tumors with Germline BRCA Mutations. *Fam. Cancer* 2009, 8, 339–346. [CrossRef]

140. Mogilyansky, E.; Clark, P.; Quann, K.; Zhou, H.; Londin, E.; Jing, Y.; Rigoutsos, I. Post-Transcriptional Regulation of BRCA2 through Interactions with MiR-19a and MiR-19b. *Front. Genet.* 2016, 7, 143. [CrossRef] [PubMed]

141. Prensner, J.R.; Chen, W.; Iyer, M.K.; Cao, Q.; Ma, T.; Han, S.; Sahu, A.; Malik, R.; Wilder-Romans, K.; Navone, N.; et al. PCAT-1, a Long Noncoding RNA, Regulates BRCA2 and Controls Homologous Recombination in Cancer. *Cancer Res.* 2014, 74, 1651–1660. [CrossRef]
142. Wan, G.; Hu, X.; Liu, Y.; Han, C.; Sood, A.K.; Calin, G.A.; Zhang, X.; Lu, X. A Novel Noncoding RNA LncRNA-JADE Connects DNA Damage Signaling to Histone H4 Acetylation. *EMBO J.* 2013, 32, 2833–2847. [CrossRef] [PubMed]

143. Wan, G.; Mathur, R.; Hu, X.; Liu, Y.; Zhang, X.; Peng, G.; Lu, X. Long Non-Coding RNA ANRIL (CDKN2B-AS) Is Induced by the ATM-E2F1 Signaling Pathway. *Cell. Signal.* 2013, 25, 1086–1095. [CrossRef] [PubMed]

144. Moncada, S. The 1991 Ulf von Euler Lecture: The l-Arginine: Nitric Oxide Pathway. *Acta Physiol. Scand.* 1992, 145, 201–227. [CrossRef] [PubMed]

145. Marletta, M.A. Nitric Oxide Synthase: Aspects Concerning Structure and Catalysis. *Cell.* 1994, 71, 676–686. [CrossRef]

146. Moncada, S. The 1991 Ulf von Euler Lecture: The l-Arginine: Nitric Oxide Pathway. *Acta Physiol. Scand.* 1992, 145, 201–227. [CrossRef] [PubMed]

147. Marletta, M.A. Nitric Oxide Synthase: Aspects Concerning Structure and Catalysis. *Cell.* 1994, 71, 676–686. [CrossRef]

148. Bogdan, C. Nitric Oxide and the Immune Response. *Nat. Immunol.* 2001, 2, 907–916. [CrossRef]

149. Aktan, F. iNOS-Mediated Nitric Oxide Production and Its Regulation. *Life Sci.* 2004, 75, 639–653. [CrossRef]

150. Mayer, B.; Hemmens, B. Biosynthesis and Action of Nitric Oxide in Mammalian Cells. *Trends Biochem. Sci.* 1997, 22, 477–481. [CrossRef]

151. Alderton, W.K.; Cooper, C.E.; Knowles, R.G. Nitric Oxide Synthases: Structure, Function and Inhibition. *Biochem. J.* 2001, 37, 593–615. [CrossRef]

152. Abu-Soud, H.M.; Yoho, L.L.; Stuehr, D.J. Calmodulin Controls Neuronal Nitric-Oxide Synthase by a Dual Mechanism. Activation of Intra- and Inter-domain Electron Transfer. *J. Biol. Chem.* 1994, 269, 32047–32050. [CrossRef]

153. Kone, B.C.; Kuncewicz, T.; Zhang, W.; Yu, Z.-Y. Protein Interactions with Nitric Oxide Synthases: Controlling the Right Time, the Right Place, and the Right Amount of Nitric Oxide. *Am. J. Physiol.-Ren. Physiol.* 2003, 285, F178–F190. [CrossRef] [PubMed]

154. Karupiah, G.; Harris, N. Inhibition of Ribonucleotide Reductase by Nitric Oxide Derived from Thionitrites: DNA Damage Signaling to Histone H4 Acetylation. *EMBO J.* 2013, 32, 2833–2847. [CrossRef] [PubMed]

155. Venema, R.C.; Sayegh, H.S.; Kent, J.D.; Harrison, D.G. Identification, Characterization, and Comparison of the Calmodulin-Binding Domains of the Endothelial and Inducible Nitric Oxide Synthases. *J. Biol. Chem.* 1996, 271, 6435–6440. [CrossRef]

156. Kwon, N.S.; Stuehr, D.J.; Nathan, C.F. Inhibition of Tumor Cell Ribonucleotide Reductase by Macrophage-Derived Nitric Oxide. *Exp. Med.* 1994, 174, 761–767. [CrossRef]

157. Mélková, Z.; Esteban, M. Inhibition of Vaccinia Virus DNA Replication by Inducible Expression of Nitric Oxide Synthase. *J. Immunol.* 1995, 155, 5711–5718.

158. Roy, B.; Lepoirre, M.; Henry, Y.; Fontecave, M. Inhibition of Ribonucleotide Reductase by Nitric Oxide Derived from Thionitriles: Reversible Modifications of Both Subunits. *Biochemistry* 1995, 34, 5411–5418. [CrossRef]

159. Karupiah, G.; Harris, N. Inhibition of Viral Replication by Nitric Oxide and Its Reversal by Ferrous Sulfate and Tricarboxylic Acid Cycle Metabolites. *J. Exp. Med.* 1995, 181, 2171–2179. [CrossRef]

160. MacMicking, J.; Xie, Q.; Nathan, C. Nitric Oxide and Macrophage Function. *Annu. Rev. Immunol.* 1997, 15, 323–350. [CrossRef]

161. Kubes, P. Inducible Nitric Oxide Synthase: A Little Bit of Good in All of Us. *Nat. Immunol.* 2001, 2, 907–916. [CrossRef]

162. Buttery, L.D.; Evans, T.J.; Springall, D.R.; Carpenter, A.; Cohen, J.; Polak, J.M. Immunological Characterization of Inducible Nitric Oxide Synthase in Endotoxic-Treated Rats. *Lab. Investig.* 1994, 71, 755–764.

163. Fang, T.; Tian, W.; Chen, G.; Li, H.; Zhang, Y.; Zhang, W.; Gao, Y.; Wang, J.; Li, S.; Wang, X.; et al. A Novel Noncoding RNA LncRNA-JADE Connects DNA Damage Signaling to Histone H4 Acetylation. *EMBO J.* 2013, 32, 2833–2847. [CrossRef] [PubMed]

164. Kubes, P. Inducible Nitric Oxide Synthase: A Little Bit of Good in All of Us. *Nat. Immunol.* 2001, 2, 907–916. [CrossRef]

165. Palinski, W.; Rosenfeld, M.E.; Ylä-Herttuala, S.; Gurtner, G.C.; Socher, S.S.; Butler, S.W.; Parthasarathy, S.; Carew, T.E.; Steinberg, D.; Witztum, J.L. Low Density Lipoprotein Undergoes Oxidative Modification in Vivo. *Proc. Natl. Acad. Sci. USA* 1994, 91, 3051–3064. [CrossRef] [PubMed]

166. Palinski, W.; Rosenfeld, M.E.; Ylä-Herttuala, S.; Gurtner, G.C.; Socher, S.S.; Butler, S.W.; Parthasarathy, S.; Carew, T.E.; Steinberg, D.; Witztum, J.L. Low Density Lipoprotein Undergoes Oxidative Modification in Vivo. *Proc. Natl. Acad. Sci. USA* 1994, 91, 3051–3064. [CrossRef] [PubMed]

167. Hu, Y.; Xiang, J.; Su, L.; Tang, X. The Regulation of Nitric Oxide in Tumor Progression and Therapy. *J. Int. Med. Res.* 2020, 48, 0300060520905985. [CrossRef]

168. Kleinert, H.; Pautz, A.; Linker, K.; Schwarz, P. M. Regulation of the Expression of Inducible Nitric Oxide Synthase. *Eur. J. Pharmacol.* 2004, 500, 255–266. [CrossRef]

169. Taylor-Robinson, A.W.; Liew, F.Y.; Severn, A.; Xu, D.; McSorley, S.J.; Garside, P.; Padron, J.; Phillips, R.S. Regulation of the Immune Response by Nitric Oxide Differentially Produced by T Helper Type 1 and T Helper Type 2 Cells. *Eur. J. Immunol.* 1994, 24, 980–984. [CrossRef]

170. Kröncke, K.-D.; Fehsel, K.; Kolb-Bachofen, V. Inducible Nitric Oxide Synthase in Human Diseases. *Clin. Exp. Immunol.* 1998, 113, 147–156. [CrossRef]

171. Chartrain, N.A.; Geller, D.A.; Kuty, P.P.; Sitrin, N.E.; Nussler, A.K.; Hoffman, E.P.; Billiar, T.R.; Hutchinson, N.I.; Mudgett, J.S. Molecular Cloning, Structure, and Chromosomal Localization of the Human Inducible Nitric Oxide Synthase Gene. *J. Biol. Chem.* 1994, 269, 6765–6772. [CrossRef]
173. Morris, S.M.; Billiar, T.R. New Insights into the Regulation of Inducible Nitric Oxide Synthesis. *Am. J. Physiol.-Endocrinol. Metab.* 1994, 266, E829–E839. [CrossRef] [PubMed]

174. Geller, D.A.; Lowenstein, C.J.; Shapiro, R.A.; Nussler, A.K.; Di Silvio, M.; Wang, S.C.; Nakayama, D.K.; Simmons, R.L.; Snyder, S.H.; Billiar, T.R. Molecular Cloning and Expression of Inducible Nitric Oxide Synthase from Human Hepatocytes. *Proc. Natl. Acad. Sci. USA* 1993, 90, 3491–3495. [CrossRef] [PubMed]

175. Kleinert, H.; Schwarz, P.M.; Förstermann, U. Regulation of the Expression of Inducible Nitric Oxide Synthase. *Biol. Chem. 2003, 384, 1343–1364. [CrossRef]*

176. Eberhardt, W.; Plüss, C.; Hummel, R.; Pfeilschifter, J. Molecular Mechanisms of Inducible Nitric Oxide Synthase Gene Expression by IL-1β and CAMP in Rat Mesangial Cells. *J. Immunol. 1998, 160, 4961–4969.*

177. Tedeschi, E.; Menegazzi, M.; Margotto, D.; Suzuki, H.; Förstermann, U.; Kleinert, H. Anti-Inflammatory Actions of St. John’s Wort: Inhibition of Human Inducible Nitric Oxide Synthase Expression by Down-Regulating Signal Transducer and Activator of Transcription-1α (STAT-1α) Activation. *J. Pharm. Exp. 2003, 307, 254–261. [CrossRef]*

178. Tedeschi, E.; Menegazzi, M.; Yao, Y.; Suzuki, H.; Förstermann, U.; Kleinert, H. Green Tea Inhibits Human Inducible Nitric-Oxide Synthase Expression by Down-Regulating Signal Transducer and Activator of Transcription-1α Activation. *Mol. Pharm. 2004, 65, 111–120. [CrossRef]*

179. Yao, Y.; Hausding, M.; Erkel, G.; Anci, T.; Förstermann, U.; Kleinert, H. Sporogen, SI4-95, and S-Curcularin, Three Inhibitors of Human Inducible-Nitric Oxide Synthase Expression Isolated from Fungi. *Mol. Pharm. 2003, 63, 383–391. [CrossRef]*

180. Eberhardt, W.; Plüss, C.; Hummel, R.; Pfeilschifter, J. Molecular Mechanisms of Inducible Nitric Oxide Synthase Gene Expression by IL-1β and CAMP in Rat Mesangial Cells. *J. Immunol. 1998, 160, 4961–4969.*

181. Goldring, C.E.P.; Reveneau, S.; Algarité, M.; Jeannin, J.-F. In Vivo Footprinting of the Mouse Inducible Nitric Oxide Synthase Gene: Inducible Protein Occupation of Numerous Sites Including Oct and NF-IL6. *Nucleic Acids Res. 1996, 24, 1682–1687. [CrossRef]*

182. Xie, Q. A Novel Lipopolysaccharide-Response Element Contributes to Induction of Nitric Oxide Synthase. *J. Biol. Chem. 1997, 272, 14867–14872. [CrossRef] [PubMed]*

183. Kim, Y.M.; Ko, C.B.; Park, Y.P.; Kim, Y.J.; Paik, S.G. Octamer Motif Is Required for the NF-kappaB-Mediated Induction of the Inducible Nitric Oxide Synthase Gene. *Mol Cells 2004, 225–260. [CrossRef]*

184. Li, M.; Pascual, G.; Glass, C.K. Peroxisome Proliferator-Activated Receptor-γ Effects in Human Chondrocytes by Inhibiting NF-KB and AP-1 Activation Pathways. *FEBS Lett. 2001, 501, 24–30. [CrossRef]*

185. Fahmi, H.; Di Battista, J.A.; Pelletier, J.-P.; Mineau, F.; Ranger, P.; Martel-Pelletier, J. Peroxisome Proliferator–Activated Receptor-γ Activators Inhibit Interleukin-1β–Induced Nitric Oxide and Matrix Metalloproteinase 13 Production in Human Chondrocytes. *Arthritis Rheum. 2001, 44, 595–607. [CrossRef][PubMed]*

186. Li, M.; Pascual, G.; Glass, C.K. Peroxisome Proliferator–Activated Receptor-γ-Dependent Repression of the Inducible Nitric Oxide Synthase Gene. *Mol. Cell. Biol. 2000, 20, 4699–4707. [CrossRef]*

187. Morris, S.M.; Billiar, T.R. Molecular Cloning and Expression of Inducible Nitric-Oxide Synthase from Human Hepatocytes. *Am. J. Physiol.-Endocrinol. Metab. 1996, 270, E829–E839. [CrossRef] [PubMed]*

188. Eberhardt, W.; Plüss, C.; Hummel, R.; Pfeilschifter, J. Molecular Mechanisms of Inducible Nitric Oxide Synthase Gene Expression by IL-1β and CAMP in Rat Mesangial Cells. *J. Immunol. 1998, 160, 4961–4969.*

189. Tedeschi, E.; Menegazzi, M.; Margotto, D.; Suzuki, H.; Förstermann, U.; Kleinert, H. Anti-Inflammatory Actions of St. John’s Wort: Inhibition of Human Inducible Nitric Oxide Synthase Expression by Down-Regulating Signal Transducer and Activator of Transcription-1α (STAT-1α) Activation. *J. Pharm. Exp. 2003, 307, 254–261. [CrossRef]*

190. Jung, F.; Palmer, L.A.; Zhou, N.; Johns, R.A. Hypoxic Regulation of Inducible Nitric Oxide Synthase via Hypoxia Inducible Factor-1 in Cardiac Myocytes. *Circ. Res. 2000, 86, 319–325. [CrossRef]*

191. Sirsjö, A.; Gidlöf, A.C.; Olsson, A.; Törmä, H.; Ares, M.; Kleinert, H.; Förstermann, U.; Hansson, G.K. Retinoic Acid Inhibits Nitric Oxide-Induced P53 Accumulation and Regulation of Inducible Nitric Oxide Synthase Expression by Wild-Type P53. *Mol. Pharmacol. 1997, 53, 4699–4707. [CrossRef]*

192. Uchimura, K.; Nakamuta, M.; Enjoji, M.; Irie, T.; Sugimoto, R.; Muta, T.; Iwamoto, H.; Nawata, H. Activation of Retinoic X Receptor and Peroxisome Proliferator–Activated Receptor-γ Inhibits Nitric Oxide and Tumor Necrosis Factor-α Production in Rat Kupffer Cells. *Hepatology 2001, 33, 91–99. [CrossRef] [PubMed]*

193. Uchimura, K.; Nakamuta, M.; Enjoji, M.; Irie, T.; Sugimoto, R.; Muta, T.; Iwamoto, H.; Nawata, H. Activation of Retinoic X Receptor and Peroxisome Proliferator–Activated Receptor-γ Inhibits Nitric Oxide and Tumor Necrosis Factor-α Production in Rat Kupffer Cells. *Hepatology 2001, 33, 91–99. [CrossRef] [PubMed]*

194. You, H.J.; Kim, J.Y.; Jeong, H.G. 17β-Estradiol Increases Inducible Nitric Oxide Synthase Expression in Macrophages. *Biochem. Biophys. Res. Commun. 2003, 303, 1129–1324. [CrossRef]*

195. Mathy, N.W.; Burleigh, O.; Kochvar, A.; Whiteford, E.R.; Behrens, M.; Marta, P.; Tian, C.; Gong, A.-Y.; Drescher, K.M.; Steyger, P.S.; et al. A Novel Long Intergenic Non-Coding RNA, Nostril, Regulates INOS Gene Transcription and Neurotoxicity in Microglia. *J. Neuroinflammation 2021, 18, 16. [CrossRef]*

196. Yamamoto, M.; Okamoto, T.; Takeda, K.; Sato, S.; Sanjo, H.; Uematsu, S.; Saitoh, T.; Yamamoto, N.; Sakurai, H.; Ishii, K.J.; et al. Key Function for the Ubc13 E2 Ubiquitin-Conjugating Enzyme in Immune Receptor Signaling. *Nat. Immunol. 2006, 7, 962–970. [CrossRef]*

197. Mathy, N.W.; Burleigh, O.; Kochvar, A.; Whiteford, E.R.; Behrens, M.; Marta, P.; Tian, C.; Gong, A.-Y.; Drescher, K.M.; Steyger, P.S.; et al. A Novel Long Intergenic Non-Coding RNA, Nostril, Regulates INOS Gene Transcription and Neurotoxicity in Microglia. *J. Neuroinflammation 2021, 18, 16. [CrossRef]*

198. Mathy, N.W.; Burleigh, O.; Kochvar, A.; Whiteford, E.R.; Behrens, M.; Marta, P.; Tian, C.; Gong, A.-Y.; Drescher, K.M.; Steyger, P.S.; et al. A Novel Long Intergenic Non-Coding RNA, Nostril, Regulates INOS Gene Transcription and Neurotoxicity in Microglia. *J. Neuroinflammation 2021, 18, 16. [CrossRef]*

199. Mathy, N.W.; Burleigh, O.; Kochvar, A.; Whiteford, E.R.; Behrens, M.; Marta, P.; Tian, C.; Gong, A.-Y.; Drescher, K.M.; Steyger, P.S.; et al. A Novel Long Intergenic Non-Coding RNA, Nostril, Regulates INOS Gene Transcription and Neurotoxicity in Microglia. *J. Neuroinflammation 2021, 18, 16. [CrossRef]*

200. Mathy, N.W.; Burleigh, O.; Kochvar, A.; Whiteford, E.R.; Behrens, M.; Marta, P.; Tian, C.; Gong, A.-Y.; Drescher, K.M.; Steyger, P.S.; et al. A Novel Long Intergenic Non-Coding RNA, Nostril, Regulates INOS Gene Transcription and Neurotoxicity in Microglia. *J. Neuroinflammation 2021, 18, 16. [CrossRef]*
197. Hayden, M.S.; Ghosh, S. Shared Principles in NF-KB Signaling. Cell 2008, 132, 344–362. [CrossRef]

198. Hu, G.; Gong, A.-Y.; Wang, Y.; Ma, S.; Chen, X.; Chen, J.; Su, C.-J.; Shibata, A.; Strauss-Soukup, J.K.; Drescher, K.M.; et al. LineRNA-Cox2 Promotes Late Inflammatory Gene Transcription in Macrophages through Modulating SWI/SNF-Mediated Chromatin Remodeling. J. Immunol. 2016, 196, 2799–2808. [CrossRef]

199. Vodovozov, Y.; Bogdan, C.; Paik, J.; Xie, Q.W.; Nathan, C. Mechanisms of Suppression of Macrophage Nitric Oxide Release by Transforming Growth Factor Beta. J. Exp. Med. 1993, 178, 605–613. [CrossRef]

200. Pautz, A.; Art, J.; Hahn, S.; Nowag, S.; Voss, C.; Kleinert, H. Regulation of the Expression of Inducible Nitric Oxide Synthase. Nitric Oxide 2010, 23, 75–93. [CrossRef]

201. Zhao, J.; Sun, B.K.; Erwin, J.A.; Song, J.-J.; Lee, J.T. Polycomb Proteins Targeted by a Short Repeat RNA to the Mouse X Chromosome. Science 2008, 322, 750–756. [CrossRef]

202. Hacisuleyman, E.; Goff, L.A.; Trapnell, C.; Williams, A.; Henao-Mejia, J.; Sun, L.; McClanahan, P.; Hendrickson, D.G.; Sauvageau, M.; Kelley, D.R.; et al. Topological Organization of Multichromosomal Regions by the Long Intergenic Noncoding RNA Firre. Nat. Struct. Mol. Biol. 2014, 21, 198–206. [CrossRef] [PubMed]

203. Zhao, T.; Cai, M.; Liu, M.; Su, G.; An, D.; Moon, B.; Lyu, G.; Si, Y.; Chen, L.; Lu, W. LncRNA 5430416N02Rik Promotes the Proliferation of Mouse Embryonic Stem Cells by Activating Mdi Expression through 3D Chromatin Architecture. Stem Cell Rep. 2020, 14, 493–505. [CrossRef] [PubMed]

204. Chan, G.C.; Fish, J.E.; Mawji, I.A.; Leung, D.D.; Rachlis, A.C.; Marsden, P.A. Epigenetic Basis for the Transcriptional Hyporesponsiveness of the Human Inducible Nitric Oxide Synthase Gene in Vascular Endothelial Cells. J. Immunol. 2005, 175, 3846–3861. [CrossRef] [PubMed]

205. Matouk, C.C.; Marsden, P.A. Epigenetic Regulation of Vascular Endothelial Gene Expression. Circ. Res. 2008, 102, 873–887. [CrossRef] [PubMed]

206. Deng, W.-G.; Wu, K.K. Regulation of Inducible Nitric Oxide Synthase Expression by P300 and P50 Acetylation. J. Immunol. 2003, 171, 6581–6588. [CrossRef]

207. Yu, Z.; Kone, B.C. Hypermethylidy of the Inducible-Nitric-Oxide-Synthase Gene Promoter Inhibits Its Transcription. J. Biol. Chem. 2004, 279, 46954–46961. [CrossRef]

208. de Andres, M.C.; Takahashi, A.; Orefio, R.O. Demethylation of an Nuclear Factor-Kb (NF-Kb) Enhancer Element Orchestrates INOS Induction in Osteoarthritis via Cell Cycle Regulation. Osteoarthr. Cartil. 2015, 23, A191. [CrossRef]

209. Linker, K.; Pautz, A.; Fechir, M.; Hubrich, T.; Greeve, J.; Kleinert, H. Involvement of KSRP in the Post-Transcriptional Regulation of Human INOS Expression—Complex Interplay of KSRP with TTP and HuR. Nucleic Acids Res. 2005, 33, 4813–4827. [CrossRef]

210. Stefano, G.B.; Salzet, M.; Magazine, H.I.; Bilfinger, T.V. Antagonism of LPS and IFN-γ Induction of INOS in Human Saphenous Vein Endothelium by Morphine and Anandamide by Nitric Oxide Inhibition of Adenylate Cyclase. J. Cardiovasc. Pharmacol. 1998, 31, 813–820. [CrossRef]

211. Stefano, G.B.; Goumon, Y.; Bilfinger, T.V.; Welters, I.D.; Cadet, P. Basal Nitric Oxide Limits Immune, Nervous and Cardiovascular Excitation: Human Endothelia Express a Mu Opiate Receptor. Prog. Neurobiol. 2000, 60, 513–530. [CrossRef]

212. Radomski, M.W.; Palmer, R.M.; Moncada, S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proc. Natl. Acad. Sci. USA 1988, 85, 6581–6588. [CrossRef] [PubMed]

213. Beckman, J.S.; Koppenol, W.H. Nitric Oxide, Superoxide, and Peroxynitrite: The Good, the Bad, and Ugly. Proc. Natl. Acad. Sci. USA 1996, 93, 14856–14863. [CrossRef] [PubMed]

214. Kelm, M. Nitric Oxide Metabolism and Breakdown. Biochem. Biophys. Acta Bioenerg. 1999, 1411, 273–289. [CrossRef]

215. Farrell, A.J.; Blake, D.R. Nitric Oxide. Ann. Rheum. Dis. 1996, 55, 7–20. [CrossRef] [PubMed]

216. Stamler, J.S.; Lamas, S.; Fang, F.C. Nitrosylation: The Prototypic Redox-Based Signaling Mechanism. Cell 2001, 106, 675–683. [CrossRef]

217. Marshall, H.E.; Hess, D.T.; Stamler, J.S. N-Nitrosylation: Physiological Regulation of NF-KB. Proc. Natl. Acad. Sci. USA 2004, 101, 8841–8842. [CrossRef] [PubMed]

218. Reyenga, N.L.; Ckless, K.; Korn, S.H.; Vos, N.; Guala, A.S.; Wouters, E.F.M.; van der Vliet, A.; Janssen-Heininger, Y.M.W.; van der Vliet, A.; Janssen-Heininger, Y.M.W.; TTP and HuR. Nucleic Acids Res. 1996, 24, 2236–2242. [CrossRef]

219. Matthews, J.R.; Botting, C.H.; Panico, M.; Morris, H.R.; Hay, R.T. Inhibition of NF-KB DNA Binding by Nitric Oxide. Nucleic Acids Res. 1996, 24, 873–887. [CrossRef]

220. Marshall, H.E.; Stamler, J.S. Inhibition of NF-KB by S-Nitrosylation. Biochemistry 2001, 40, 1688–1693. [CrossRef]

221. King, M.-C.; Marks, J.H.; Mandell, J.B. Breast and Ovarian Cancer Risks Due to Inherited Mutations in BRCA1 and BRCA2. Science 2003, 302, 643–646. [CrossRef]

222. Saad, A.F.; Hu, W.; Sood, A.K. Microenvironment and Pathogenesis of Epithelial Ovarian Cancer. Horm. Cancer 2010, 1, 277–290. [CrossRef] [PubMed]

223. Malone, J.M.; Saed, G.M.; Diamond, M.P.; Sokol, R.J.; Munkarah, A.R. The Effects of the Inhibition of Inducible Nitric Oxide Synthase on Angiogenesis of Epithelial Ovarian Cancer. Am. J. Obstet. Gynecol. 2006, 194, 1110–1116. [CrossRef] [PubMed]

224. Saed, G.M.; Ali-Fehmi, R.; Jiang, Z.L.; Fletcher, N.M.; Abu-Soud, H.M.; Munkarah, A.R. Myeloperoxidase Serves as a Redox Switch That Regulates Apoptosis in Epithelial Ovarian Cancer. Gynecol. Oncol. 2010, 116, 276–281. [CrossRef] [PubMed]
Antioxidants 2022, 11, 1195

225. Jiang, Z.; Fletcher, N.M.; Ali-Fehmi, R.; Diamond, M.P.; Abu-Soud, H.M.; Munkarah, A.R.; Saed, G.M. Modulation of Redox Signaling Promotes Apoptosis in Epithelial Ovarian Cancer Cells. *Gynecol. Oncol.* 2011, 122, 418–423. [CrossRef]

226. Saed, G.M.; Diamond, M.P.; Fletcher, N.M. Updates of the Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer. *Gynecol. Oncol.* 2017, 145, 595–602. [CrossRef]

227. Sha, Y.; Marshall, H.E. S-Nitrosylation in the Regulation of Gene Transcription. *Biochim. Biophys. Acta Gen. Subj.* 2012, 1820, 701–711. [CrossRef]

228. van Dieck, J.; Teufel, D.P.; Jaulent, A.M.; Fernandez-Fernandez, M.R.; Rutherford, T.J.; Wyszluch-Czyszynska, A.; Ferhst, A.R. Posttranslational Modifications Affect the Interaction of S100 Proteins with Tumor Suppressor P53. *J. Mol. Biol.* 2009, 394, 922–930. [CrossRef]

229. Schonhoff, C.M.; Daou, M.-C.; Jones, S.N.; Schiffer, C.A.; Ross, A.H. Nitric Oxide-Mediated Inhibition of Hdm2–p53 Binding. *Biochemistry* 2002, 41, 13570–13574. [CrossRef]

230. Padgett, C.M.; Whorton, A.R. S-Nitrosoglutathione Reversibly Inhibits GAPDH by S-Nitrosylation. *Am. J. Physiol.-Cell Physiol.* 1995, 269, C739–C749. [CrossRef]

231. Jia, J.; Arif, A.; Willard, B.; Plow, E.F.; Hazen, S.L.; Fox, P.L. Target-Selective Protein S-Nitrosylation by Sequence Motif Recognition. *Cell 2014*, 159, 623–634. [CrossRef]

232. Jia, J.; Arif, A.; Willard, B.; Smith, J.D.; Stuehr, D.J.; Hazen, S.L.; Fox, P.L. Protection of Extraribosomal RPL13a by GAPDH and Dysregulation by S-Nitrosylation. *Mol. Cell 2012*, 47, 656–663. [CrossRef] [PubMed]

233. Lehmann, B.D.; Bauer, J.A.; Chen, X.; Sanders, M.E.; Chakravarthy, A.B.; Shyr, Y.; Pietenpol, J.A. Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies. *J. Clin. Investig.* 2011, 121, 2750–2767. [CrossRef] [PubMed]

234. Thomas, D.D.; Ridnour, L.A.; Isenberg, J.S.; Flores-Santana, W.; Switzer, C.H.; Donzelli, S.; Hussain, P.; Vecoli, C.; Paolocci, N.; Amb, S.; et al. The Chemical Biology of Nitric Oxide: Implications in Cell Survival and Signaling. *Free Radic. Biol. Med.* 2008, 45, 18–31. [CrossRef] [PubMed]

235. Wink, D.A.; Hines, H.B.; Cheng, R.Y.S.; Switzer, C.H.; Flores-Santana, W.; Vitek, M.P.; Ridnour, L.A.; Colton, C.A. Nitric Oxide and Redox Mechanisms in the Immune Response. *J. Leukoc. Biol.* 2011, 89, 873–891. [CrossRef] [PubMed]

236. Glynn, S.A.; Boersma, B.J.; Dorsey, T.H.; Yi, M.; Yang, H.G.; Ridnour, L.A.; Martin, D.N.; Switzer, C.H.; Hudson, R.S.; Wink, D.A.; et al. Increased NOS2 Predicts Poor Survival in Estrogen Receptor–Negative Breast Cancer Patients. *J. Clin. Investig.* 2010, 120, 3843–3854. [CrossRef]

237. Switzer, C.H.; Cheng, R.Y.S.; Ridnour, L.A.; Glynn, S.A.; Amb, S.; Wink, D.A. Ets-1 Is a Transcriptional Mediator of Oncogenic Nitric Oxide Signaling in Estrogen Receptor-Negative Breast Cancer. *Breast Cancer Res. 2012*, 14, R125. [CrossRef]

238. Tsuji, M.; Kawano, S.; Tsuji, S.; Sawaoka, H.; Hori, M.; DuBois, R.N. Cyclooxygenase Regulates Angiogenesis Induced by Colon Cancer Cells. *Cell 1998*, 93, 705–716. [CrossRef] [PubMed]

239. Brown, J.R.; DuBois, R.N. COX-2: A Molecular Target for Colorectal Cancer Prevention. *J. Clin. Oncol.* 2005, 23, 2840–2855. [CrossRef]

240. Wu, Y.; Zhou, B.P. Inflammation: A Driving Force Speeds Cancer Metastasis. *Cell Cycle 2009*, 8, 3267–3273. [CrossRef]

241. Grellner, W.; Georg, T.; Wilske, J. Quantitative Analysis of Proinflammatory Cytokines (IL-1β, IL-6, TNFα) in Human Skin Wounds. *Forensic Sci. Int.* 2009, 193, 251–264. [CrossRef]

242. Balkwill, F.; Mantovani, A. Inflammation and Cancer: Cancer to Back to Virchow? *Lancet 2001*, 357, 539–545. [CrossRef]

243. Zidi, I.; Mestiri, S.; Bartegi, A.; Amor, N.B. TNF-α and Its Inhibitors in Cancer. *Med. Oncol.* 2010, 27, 185–198. [CrossRef] [PubMed]

244. Fernando, R.I.; Castillo, M.D.; Litzinger, M.; Hamilton, D.H.; Palena, C. IL-8 Signaling Plays a Critical Role in the Epithelial–Mesenchymal Transition of Human Carcinoma Cells. *Cancer Res. 2011*, 71, 5296–5306. [CrossRef] [PubMed]

245. Mantovani, A.; Bonecchi, R.; Locati, M. Tuning Inflammation and Immunity by Chemokine Sequestration: Decoys and More. *Nat. Rev. Immunol.* 2006, 6, 907–918. [CrossRef] [PubMed]

246. Balkwill, F. Tumour Necrosis Factor and Cancer. *Nat. Rev. Cancer 2009*, 9, 361–371. [CrossRef]

247. Kaler, P.; Augenlicht, L.; Klampfer, L. Macrophage-Derived IL-1β Stimulates Wnt Signaling and Growth of Colon Cancer Cells: A Crossstalk Interrupted by Vitamin D3. *Oncogene 2009*, 28, 3892–3902. [CrossRef]

248. Ben-Baruch, A. The Tumor-Promoting Flow of Cells Into, Within and Out of the Tumor Site: Regulation by the Inflammatory Axis of TNFα and Chemokines. *Cancer Microenvir.* 2012, 5, 151–164. [CrossRef]

249. Granados-Principal, S.; Liu, Y.; Guevara, M.L.; Blanco, E.; Choi, D.S.; Qian, W.; Patel, T.; Rodriguez, A.A.; Cusimano, J.; Weiss, H.L.; et al. Inhibition of INOS as a Novel Effective Targeted Therapy against Triple-Negative Breast Cancer. *Breast Cancer Res. 2015*, 17, 25. [CrossRef]

250. Eyler, C.E.; Wu, Q.; Yan, K.; MacSwords, J.M.; Chandler-Militello, D.; Misuraca, L.C.; Lathia, J.D.; Forrester, M.T.; Lee, J.; Stamler, J.S.; et al. Glialoma Stem Cell Proliferation and Tumor Growth Are Promoted by Nitric Oxide Synthase-2. *Cell 2011*, 146, 53–66. [CrossRef] [PubMed]

251. Okayama, H.; Saito, M.; Oue, N.; Weiss, J.M.; Stauffer, J.; Takenoshita, S.; Wiltout, R.H.; Hussain, S.P.; Harris, C.C. NOS2 Enhances KRAS-Induced Lung Carcinogenesis, Inflammation and MicroRNA-21 Expression. *Int. J. Cancer 2013*, 132, 9–18. [CrossRef] [PubMed]

252. Dittmer, J. The Biology of the Ets1 Proto-Oncogene. *Mol. Cancer 2003*, 2, 29. [CrossRef] [PubMed]

253. Lincoln, D.W., 2nd; Bove, K. The Transcription Factor Ets-1 in Breast Cancer. *Front. Biosci.-Landmark 2005*, 10, 506–511. [CrossRef] [PubMed]
Antioxidants 2022, 11, 1195

254. Turner, D.P.; Findlay, V.J.; Moussa, O.; Watson, D.K. Defining ETS Transcription Regulatory Networks and Their Contribution to Breast Cancer Progression. J. Cell. Biochem. 2007, 102, 549–559. [CrossRef] [PubMed]

255. Nazir, S.U.; Kumar, R.; Singh, A.; Khan, A.; Tanwar, P.; Tripathi, R.; Mehrotra, R.; Hussain, S. Breast Cancer Invasion and Progression by MMP-9 through Ets-1 Transcription Factor. Gene 2019, 711, 143952. [CrossRef] [PubMed]

256. Park, Y.H.; Jung, H.H.; Ahn, J.S.; Im, Y.H. Ets-1 Upregulates HER2-Induced MMP-1 Expression in Breast Cancer Cells. Biochem. Biophys. Res. Commun. 2008, 377, 389–394. [CrossRef]

257. Kim, S.; Han, J.; Shin, I.; Kil, W.H.; Lee, J.E.; Nam, S.J. A Functional Comparison between the HER2high/HER3 and the HER2low/HER3 Dimers on Heregulin-B1-Induced MMP-1 and MMP-9 Expression in Breast Cancer Cells. Exp. Mol. Med. 2012, 44, 473–482. [CrossRef]

258. Haynes, M.P.; Li, L.; Sinha, D.; Russell, K.S.; Hisamoto, K.; Baron, R.; Collinge, M.; Sessa, W.C.; Bender, J.R. Src Kinase Mediates Phosphatidylinositol 3-Kinase/Akt-Dependent Rapid Endothelial Nitric-Oxide Synthase Activation by Estrogen. J. Biol. Chem. 2003, 278, 2118–2123. [CrossRef]

259. Switzer, C.H.; Glynn, S.A.; Ridnour, L.A.; Cheng, R.Y.-S.; Vitek, M.P.; Amb, S.; Wink, D.A. Nitric Oxide and Protein Phosphatase 2A Provide Novel Therapeutic Opportunities in ER-Negative Breast Cancer. Trends Pharmacol. Sci. 2011, 32, 644–651. [CrossRef]

260. Wink, D.A.; Kasprzak, K.S.; Maragos, C.M.; Elespuru, R.K.; Misra, M.; Dunams, T.M.; Cebula, T.A.; Koch, W.H.; Andrews, A.W.; Allen, J.S.; et al. DNA Deaminating Ability and Genotoxicity of Nitric Oxide and Its Progenitors. Science 1991, 254, 1001–1003. [CrossRef]

261. Nguyen, T.; Brunson, D.; Crespi, C.L.; Penman, B.W.; Wishnok, J.S.; Tannenbaum, S.R. DNA Damage and Mutation in Human Cells Exposed to Nitric Oxide in Vitro. Proc. Natl. Acad. Sci. USA 1992, 89, 3030–3034. [CrossRef]

262. Jaiswal, M.; LaRusso, N.F.; Burgart, L.J.; Gores, G.J. Inflammatory Cytokines Induce DNA Damage and Inhibit DNA Repair in Cholangiocarcinoma Cells by a Nitric Oxide-Dependent Mechanism I. Cancer Res. 2000, 60, 184–190. [PubMed]

263. Jaiswal, M.; LaRusso, N.F.; Shapiro, R.A.; Billadeau, D.D.; Kaufmann, S.H.; Li, H. Uncovering Pharmacological Opportunities for Cancer Stem Cells—A Systems Biology View. Front. Cell Dev. Biol. 2022, 10, 752326. [CrossRef] [PubMed]

264. Rieder, J.; Jahnke, R.; Schloesser, M.; Seibel, M.; Czechowski, M.; Marth, C.; Hoffmann, G. Nitric Oxide-Dependent Apoptosis in Ovarian Carcinoma Cells. Gynecol. Oncol. 2004, 91, 172–176. [CrossRef]

265. Miki, Y.; Swensen, J.; Shattuck-Eidens, D.; Futreal, P.A.; Harshman, K.; Tavtigian, S.; Liu, Q.; Cochran, C.; Bennett, L.M.; Ding, W.; et al. A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene BRCA1. Cancer Res. 1994, 2005, 33, 2181–2191. [CrossRef] [PubMed]

266. Marth, C.; Fieg, H.; Zeimet, A.G.; Müller-Holzner, E.; Deibl, M.; Doppler, W.; Daxenbichler, G. Interferon-γ Expression Is an Independent Prognostic Factor in Ovarian Cancer. Am. J. Obstet. Gynecol. 2004, 191, 1598–1605. [CrossRef]

267. Marth, C.; Müller-Holzner, E.; Greiter, E.; Cronauer, M.V.; Zeimet, A.G.; Doppler, W.; Eibl, B.; Hynes, N.E.; Daxenbichler, G. γ-Interferon Reduces Expression of the Protooncogene c-ErbB-2 in Human Ovarian Carcinoma Cells. Cancer Res. 1990, 50, 7037–7041. [CrossRef] [PubMed]

268. Gastron, R.W.; Taylor, B.S.; Shao, L.; Geller, D.A. Complex Regulation of Human Inducible Nitric Oxide Synthase Gene Transcription by Stat 1 and NF-KB. Proc. Natl. Acad. Sci. USA 2001, 98, 8638–8643. [CrossRef]

269. Rieder, J.; Jahnke, R.; Scholeser, M.; Seibel, M.; Czechowski, M.; Marth, C.; Hoffmann, G. Nitric Oxide-Dependent Apoptosis in Ovarian Carcinoma Cells. Gynecol. Oncol. 2004, 91, 172–176. [CrossRef]

270. Miki, Y.; Swensen, J.; Shattuck-Eidens, D.; Futreal, P.A.; Harshman, K.; Tavtigian, S.; Liu, Q.; Cochran, C.; Bennett, L.M.; Ding, W.; et al. A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene BRCA1. Cancer Res. 1994, 2005, 33, 2181–2191. [CrossRef] [PubMed]

271. Jaiswal, M.; LaRusso, N.F.; Burgart, L.J.; Gores, G.J. Inflammatory Cytokines Induce DNA Damage and Inhibit DNA Repair in Cholangiocarcinoma Cells by a Nitric Oxide-Dependent Mechanism I. Cancer Res. 2000, 60, 184–190. [PubMed]

272. Jaiswal, M.; LaRusso, N.F.; Shapiro, R.A.; Billadeau, D.D.; Kaufmann, S.H.; Li, H. Uncovering Pharmacological Opportunities for Cancer Stem Cells—A Systems Biology View. Front. Cell Dev. Biol. 2022, 10, 752326. [CrossRef] [PubMed]

273. Rieder, J.; Jahnke, R.; Schloesser, M.; Seibel, M.; Czechowski, M.; Marth, C.; Hoffmann, G. Nitric Oxide-Dependent Apoptosis in Ovarian Carcinoma Cells. Gynecol. Oncol. 2004, 91, 172–176. [CrossRef]

274. Le Page, F.; Randrianarison, V.; Marot, D.; Cabannes, J.; Perricaudet, M.; Feunteun, J.; Sarasin, A. BRCA1 and BRCA2 Are Necessary for the Transcription-Coupled Repair of the Oxidative 8-Oxoguanine Lesion in Human Cells I. Cancer Res. 2000, 60, 5548–5552. [PubMed]

275. Correia, C.; Weiskittel, T.M.; Ung, C.Y.; Villasboas Bisterno, J.C.; Billadeau, D.D.; Kaufmann, S.H.; Li, H. Uncovering Pharmacological Opportunities for Cancer Stem Cells—A Systems Biology View. Front. Cell Dev. Biol. 2022, 10, 752326. [CrossRef] [PubMed]

276. Yongsanguanchai, N.; Pongrakhananon, V.; Mutirangura, A.; Rojanasakul, Y.; Chanvorachote, P. Nitric Oxide Induces Cancer Stem Cell-like Phenotypes in Human Lung Cancer Cells. Am. J. Physiol.-Cell Physiol. 2015, 308, C89–C100. [CrossRef]

277. Wang, R.; Li, Y.; Tsung, A.; Huang, H.; Du, Q.; Yang, M.; Deng, M.; Xiong, S.; Wang, X.; Zhang, L.; et al. INOS Promotes CD24+CD133+ Liver Cancer Stem Cell Phenotype through a TACE/ADAM17-Dependent Notch Signaling Pathway. Proc. Natl. Acad. Sci. USA 2018, 115, E10127–E10136. [CrossRef]
278. Maiuthed, A.; Chantarawong, W.; Chanvorachote, P. Lung Cancer Stem Cells and Cancer Stem Cell-Targeting Natural Compounds. Anticancer Res. 2018, 38, 3797–3809. [CrossRef]

279. Ekmeckicoglou, S.; Grimm, E.A.; Roszk, J. Targeting INOS to Increase Efficacy of Immunotherapies. Hum. Vaccines Immunother. 2017, 13, 1105–1108. [CrossRef]

280. López-Sánchez, L.M.; Mena, R.; Guil-Luna, S.; Mantrana, A.; Peñarando, J.; Toledano-Fonseca, M.; Conde, F.; De la Haba-Rodríguez, J.R.; Aranda, E.; Rodríguez-Arizá, A. Nitric Oxide-Targeted Therapy inhibits Stemness and Increases the Efficacy of Tamoxifen in Estrogen Receptor-Positive Breast Cancer Cells. Lab. Invesit. 2021, 101, 292–303. [CrossRef]

281. Chen, Y.; Wang, W.; Wu, W.; Hsu, C.; Wei, L.; Wang, S.; Hsu, Y.; Liaw, C.; Tsai, W. Novel Histone Deacetylase Inhibitor AR-42 Exhibits Antitumor Activity in Pancreatic Cancer Cells by Affecting Multiple Biochemical Pathways. PLoS ONE 2017, 12, e0183368. [CrossRef]

282. Peñarando, J.; López-Sánchez, L.M.; Mena, R.; Guil-Luna, S.; Conde, F.; Hernández, V.; Toledano, M.; Gudiño, V.; Raponi, M.; Billard, C.; et al. A Role for Endothelial Nitric Oxide Synthase in Intestinal Stem Cell Proliferation and Mesenchymal Colorectal Cancer. BMC Biol. 2018, 16, 3. [CrossRef]

283. Kim, H.; Lin, Q.; Yun, Z. BRCA1 Regulates the Cancer Stem Cell Fate of Breast Cancer Cells in the Context of Hypoxia and Histone Deacetylase Inhibitors. Sci. Rep. 2019, 9, 9702. [CrossRef] [PubMed]

284. Pupa, S.M.; Ligorio, F.; Cancila, V.; Franceschini, A.; Tripodo, C.; Vernieri, C.; Castagnoli, L. HER2 Signaling and Breast Cancer Stem Cells: The Bridge behind HER2-Positive Breast Cancer Aggressiveness and Therapy Refractoriness. Cancers 2021, 13, 4778. [CrossRef] [PubMed]

285. Vietri, M.T.; D’Elia, G.; Caliendo, G.; Albanese, L.; Signoriello, G.; Napoli, C.; Molinari, A.M. Pancreatic Cancer with Mutation in BRCA1/2, MLH1, and APC Genes: Phenotype Correlation and Detection of a Novel Germline BRCA2 Mutation. Genes 2022, 13, 321. [CrossRef]

286. Han, S.-H.; Ryu, K.H.; Kwon, A.-Y. The Prognostic Impact of HER2 Genetic and Protein Expression in Pancreatic Carcinoma-HER2 Protein and Gene in Pancreatic Cancer. Diagnostics 2021, 11, 653. [CrossRef]

287. Porcelli, L.; Quatrale, A.E.; Mantuano, P.; Leo, M.G.; Silvestris, N.; Rolland, J.F.; Carioggia, E.; Lioce, M.; Paradiso, A.; Azzariti, A. Optimize Radiochemotherapy in Pancreatic Cancer: PARP Inhibitors a New Therapeutic Opportunity. Mol. Oncol. 2013, 7, 308–322. [CrossRef]

288. Komoto, M.; Nakata, B.; Nishii, T.; Kawajiri, H.; Shinto, O.; Amano, R.; Yamada, N.; Yashiro, M.; Hirakawa, K. In Vivo and in Vivo Evidence That a Combination of Lapatinib plus S-1 Is a Promising Treatment for Pancreatic Cancer. Cancer Sci. 2010, 101, 468–473. [CrossRef] [PubMed]

289. D’Argenio, V. The High-Throughput Analyses Era: Are We Ready for the Data Struggle? High-Throughput 2018, 7, 8. [CrossRef]

290. The Cancer Genome Atlas Research Network; Weinstein, J.N.; Collisson, E.A.; Mills, G.B.; Shaw, K.R.M.; Ozenberger, B.A.; Ellrott, K.; Shmulevich, I.; Sander, C.; Stuart, J.M. The Cancer Genome Atlas Pan-Cancer Analysis Project. Nat. Genet. 2013, 45, 1113–1120. [CrossRef]

291. Zhao, N.; Guo, M.; Wang, K.; Zhang, C.; Liu, X. Identification of Pan-Cancer Prognostic Biomarkers through Integration of Multi-Omics Data. Front. Bioeng. Biotechnol. 2020, 8, 268. [CrossRef]

292. Candido, S.; Tomasellos, B.M.R.; Lavore, A.; Falzone, L.; Gottuso, G.; Libra, M. Novel Insights into Epigenetic Regulation of Il6 Pathway: In Silico Perspective on Inflammation and Cancer Relationship. Int. J. Mol. Sci. 2021, 22, 10172. [CrossRef]

293. Zheng, Q.; Min, S.; Zhou, Q. Identification of Potential Diagnostic and Prognostic Biomarkers for LUAD Based on TCGA and GEO Databases. Biosci. Rep. 2021, 41, BSR20204370. [CrossRef] [PubMed]

294. Goldman, M.; Craft, B.; Hastie, M.; Repečka, K.; McDade, F.; Kamath, A.; Banerjee, A.; Luo, Y.; Rogers, D.; Brooks, A.N.; et al. The UCSC Xena Platform for Public and Private Cancer Genomics Data Visualization and Interpretation. bioRxiv 2019, 326470. [CrossRef]

295. Schleicher, M.; Brundin, F.; Gross, S.; Mühler-Esterl, W.; Oess, S. Cell Cycle-Regulated Inactivation of Endothelial NO Synthase through NOSIP-Dependent Targeting to the Cytoskeleton. Mol. Cell. Biol. 2005, 25, 8251–8258. [CrossRef] [PubMed]

296. Freudenberg, F.; Alttoa, A.; Reif, A. Neuronal Nitric Oxide Synthase (NOS1) and Its Adaptor, NOS1AP, as a Genetic Risk Factors for Psychiatric Disorders. Genes Brain Behav. 2015, 14, 46–63. [CrossRef]

297. Ekmekcioglu, S.; Grimm, E.A.; Roszk, J. Targeting INOS to Increase Efficacy of Immunotherapies. Hum. Vaccines Immunother. 2017, 13, 1105–1108. [CrossRef]

298. Basudhar, D.; Somasundaram, V.; de Oliveira, G.A.; Kesarwala, A.; Heinecke, J.L.; Cheng, R.Y.; Glynn, S.A.; Ambs, S.; Wink, D.A.; Ridnour, L.A. Nitric Oxide Synthase-2-Derived Nitric Oxide Drives Multiple Pathways of Breast Cancer Progression. Cell Cycle 2017, 9702. [CrossRef] [PubMed]

299. Lechner, M.; Lirk, P.; Rieder, J. Inducible Nitric Oxide Synthase (iNOS) in Tumor Biology: The Two Sides of the Same Coin. Semin. Cancer Biol. 2005, 15, 277–289. [CrossRef] [PubMed]

300. Walsh, E.M.; Keane, M.M.; Wink, D.A.; Callagy, G.; Glynn, S.A. Review of Triple Negative Breast Cancer and the Impact of Inducible Nitric Oxide Synthase on Tumor Biology and Patient Outcomes. CRO 2016, 21, 333–351. [CrossRef]

301. Foulkes, W.D. BRCA1 Functions as a Breast Stem Cell Regulator. J. Med. Genet. 2004, 41, 1–5. [CrossRef]

302. Belgorosky, D.; Girouard, J.; Langle, Y.; Hamelin Morrisette, J.; Marino, L.; Agüero, E.; Malagrino, H.; Reyes-Moreno, C.; Eiján, A.M. Relevance of iNOS Expression in Tumor Growth and Maintenance of Cancer Stem Cells in a Bladder Cancer Model. J. Mol. Med. 2020, 98, 1615–1627. [CrossRef]
303. Li, X.; Zou, Z.; Tang, J.; Zheng, Y.; Liu, Y.; Luo, Y.; Liu, Q.; Wang, Y. NOS1 Upregulates ABCG2 Expression Contributing to DDP Chemoresistance in Ovarian Cancer Cells. *Oncol. Lett.* **2019**, *17*, 1595–1602. [CrossRef] [PubMed]

304. Zou, Z.; Li, X.; Sun, Y.; Li, L.; Zhang, Q.; Zhu, L.; Zhong, Z.; Wang, M.; Wang, Q.; Liu, Z.; et al. NOS1 Expression Promotes Proliferation and Invasion and Enhances Chemoresistance in Ovarian Cancer. *Oncol. Lett.* **2020**, *19*, 2989–2995. [CrossRef] [PubMed]

305. Xu, P.; Ye, S.; Li, K.; Huang, M.; Wang, Q.; Zeng, S.; Chen, X.; Gao, W.; Chen, J.; Zhang, Q.; et al. NOS1 Inhibits the Interferon Response of Cancer Cells by S-Nitrosylation of HDAC2. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 483. [CrossRef] [PubMed]

306. Chen, X.; Zou, Z.; Wang, Q.; Gao, W.; Zeng, S.; Ye, S.; Xu, P.; Huang, M.; Li, K.; Chen, J.; et al. Inhibition of NOS1 Promotes the Interferon Response of Melanoma Cells. *J. Transl. Med.* **2022**, *20*, 205. [CrossRef]

307. Anastas, J.N.; Biechele, T.L.; Robitaille, M.; Muster, J.; Allison, K.H.; Angers, S.; Moon, R.T. A Protein Complex of SCRIB, NOS1AP and VANGL1 Regulates Cell Polarity and Migration, and Is Associated with Breast Cancer Progression. *Oncogene* **2012**, *31*, 3696–3708. [CrossRef]

308. Li, L.-L.; de Mera, R.M.M.-F.; Chen, J.; Ba, W.; Kasri, N.N.; Zhang, M.; Courtney, M.J. Unexpected Heterodivalent Recruitment of NOS1AP to NNOS Reveals Multiple Sites for Pharmacological Intervention in Neuronal Disease Models. *J. Neurosci.* **2015**, *35*, 7349–7364. [CrossRef]

309. Roselló-Lleti, E.; Tarazon, E.; Ortega, A.; Gil-Cayuela, C.; Carnicer, R.; Lago, F.; González-Juanatey, J.R.; Portolés, M.; Rivera, M. Protein Inhibitor of NOS1 Plays a Central Role in the Regulation of NOS1 Activity in Human Dilated Hearts. *Sci. Rep.* **2016**, *6*, 30902. [CrossRef]

310. Sadaf, S.; Nagarkoti, S.; Awasthi, D.; Singh, A.K.; Srivastava, R.N.; Kumar, S.; Barthwal, M.K.; Dikshit, M. NNOS Induction and NOSIP Interaction Impact Granulopoiesis and Neutrophil Differentiation by Modulating Nitric Oxide Generation. *Biochim. Biophys. Acta Mol. Cell Res.* **2021**, *1868*, 119018. [CrossRef]