HIF-1α Overexpression in Ductal Carcinoma In Situ of the Breast in BRCA1 and BRCA2 Mutation Carriers

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Abstract
Recent studies have revealed that BRCA1 and BRCA2 germline mutation-related breast cancers show frequent overexpression of hypoxia inducible factor-1α (HIF-1α), the key regulator of the hypoxia response. However, the question remained whether hypoxia is a late stage bystander or a true carcinogenetic event in patients with hereditary predisposition. We therefore studied HIF-1α overexpression in ductal carcinoma in situ (DCIS), an established precursor of invasive breast cancer. We used immunohistochemistry to examine the expression of the hypoxia markers HIF-1α, CAIX and Glut-1 in DCIS and available invasive carcinoma lesions of 32 BRCA1, 16 BRCA2 and 77 non-BRCA mutation-related cases. HIF-1α expression was detected in 63% of BRCA1 and 62% of BRCA2 as compared to 34% of non-BRCA mutation-related DCIS cases (p = 0.005). CAIX overexpression was present in 56% of BRCA1 and 44% of BRCA2 as compared to 6% of non-BRCA mutation-related DCIS cases (p = 0.005). CAIX overexpression was observed in 59% of BRCA1, 75% of BRCA2 and 67% of non-BRCA mutation-related DCIS cases (p = 0.527). Overall, HIF-1α, CAIX and Glut-1 expression in BRCA mutation-related DCIS matched the expression in the accompanying invasive cancers in 60% or more of cases. In non-BRCA mutation-related cases the expression of the hypoxia markers in DCIS matched the expression in the invasive part in 46% or more of the cases. Although BRCA1 and BRCA2 germline mutation-related invasive breast cancers are different in many ways, the hypoxia-related proteins HIF-1α, CAIX and Glut-1 are expressed in both DCIS and invasive lesions of BRCA1 and BRCA2 mutation carriers. This suggests that hypoxia may already play a role in the DCIS stage of BRCA1 and BRCA2 germline mutation related breast carcinogenesis, and may also drive cancer progression. Hypoxia-related proteins are therefore putative targets for therapy and molecular imaging for early detection and monitoring therapy response in BRCA mutation patients.

Introduction
Hereditary breast cancer accounts for about 5% of all breast cancers in women and is primarily caused by a germline mutation in one of the BRCA genes. Several studies have indicated that the genetic makeup of BRCA1 and BRCA2 mutation-related breast cancer is different from that of non-BRCA1 mutation-related breast cancer. These differences comprise gains and losses of specific parts of chromosomes, as well as differences in protein expression [1–7]. Consistent with this, the morphological and immunohistochemical phenotype of BRCA1 mutation-related breast cancer is also different from that of non-BRCA1 mutation-related breast cancer [8–15]. However, the phenotype of BRCA2 mutation-related breast cancer is still difficult to distinguish from non-BRCA1 mutation-related breast cancers [14,15].

Hypoxia is a hallmark of many non-BRCA mutation-related breast cancer types [16]. Hypoxia inducible factor-1 (HIF-1) is the key regulator of the hypoxia response. HIF-1 consists of 2 subunits, HIF-1α and HIF-1β. While HIF-1β is constitutively expressed, the HIF-1α protein is continuously degraded under normoxia by the ubiquitin-proteasome pathway [17,18]. Under hypoxia, HIF-1α protein degradation is inhibited resulting in its overexpression, subsequent binding to HIF-1β [19] and downstream signalling [20]. In non-BRCA mutation-related breast cancer, HIF-1α overexpression plays a role in carcinogenesis [21–26] and correlates with poor prognosis [27,28]. When HIF-1α is overexpressed, established downstream targets like Carbonic anhydrase IX (CAIX) and Glucose transporter-1 (Glut-1) are also up regulated [29,30]. BRCA1 seems to play a role in the hypoxic response by regulating HIF-1α stability and by modulating expression of vascular endothelial growth factor, a major downstream target of HIF-1α [31]. Furthermore, functional HIF-1α overexpression (mostly hypoxia induced) is seen at a much higher frequency in BRCA1 mutation-related invasive breast cancer than in sporadic breast cancer [32,33]. In contrast, BRCA2

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mutation-related invasive cancers express HIF-1α less frequently [33].

However, studies in pre-invasive lesions are required to address the question whether hypoxia is a late stage bystander or a true carcinogenetic event.

There is both clinical and experimental evidence to suggest that ductal carcinoma in situ (DCIS) is a precursor lesion to most, if not all, non-BRCA mutation-related invasive breast cancers [34–39]. DCIS and other premalignant lesions such as lobular neoplasia, fibroadenoma, and ductal hyperplasia seems to be more common in prophylactic mastectomy (PM) specimens of BRCA1 and BRCA2 mutation carriers than in control mammoplasty specimens [10,39–42]. Furthermore, DCIS lesions adjacent to invasive cancers in BRCA1 mutation carriers have been described [43,44]. DCIS in BRCA1 mutation carriers is often high grade [43] and shows a similar morphology and immunophenotype as the accompanying invasive cancer [45]. High grade DCIS of non-BRCA-related cases often shows central necrosis [46] indicative of hypoxia. Indeed, overexpression of hypoxia-related proteins HIF-1α, CAIX and Glut-1 DCIS of non-BRCA1 mutation carriers has been described [22]. To find clues whether changes in hypoxia related proteins also is an early event in BRCA1 mutation-related carcinogenesis, we evaluated HIF-1α expression in BRCA1 and BRCA2 mutation-related DCIS in relation with the accompanying invasive cancers.

Materials and Methods

Patients

The study group comprised DCIS lesions of 32 patients with pathogenic germline BRCA1 mutations, 16 patients with pathogenic germline BRCA2 mutations and 77 patients unselected for family history (further denoted “non-BRCA mutation-related”). A synchronous invasive tumor was also present in 28 BRCA1, 17 BRCA2 and 50 non-BRCA1 mutation-related cases. Tissue from these patients was available from our own archives, and from different pathology laboratories in The Netherlands (St Antonius Hospital Nieuwegein, Diakonessenhuis Utrecht, Gelre Ziekenhuis Apeldoorn, Rijnstate Arnhem, Stichting Pathologisch en Cytologisch laboratorium West Brabant Bergen op Zoom, Ziekenhuis Gelderse Vallei Ede, Deventer Ziekenhuis Deventer, Meander medisch centrum Amersfoort, Onze Lieve Vrouwe Gasthuis Amsterdam, the VU University Medical Center, Amsterdam and the University Medical Center Groningen). Since we used archival pathology material which does not interfere with patient care and does not involve the physical involvement of the patient, no ethical approval is required according to Dutch legislation [the Medical Research Involving Human Subjects Act (Wet medisch-wetenschappelijk onderzoek met mensen, WMO [47])] and use of anonymous or coded left over material for scientific purposes is part of the standard treatment contract with patients and therefore informed consent procedure was not required according to our institutional medical ethical review board. This has also been described by van Diest et al. [48].

Histopathology

Tumor size was measured in the fresh resection specimens, and tumor samples were subsequently fixed in neutral buffered formaldehyde, and processed to paraffin blocks according to standard procedures. Four µm thick sections were cut and stained with H&E for histopathology. Tumor type was assessed according to the WHO 2003, and tumors were graded according to the Nottingham grading system. Mitoses counting was performed as previously described [49]. Scoring was performed by one observer (PJvdD) who was blinded to the origin of the tumors.

Immunohistochemistry

After deparaffinization and rehydration, antigen retrieval was performed using EDTA buffer at boiling temperature for 20 minutes for ER, HER2 and HIF-1α. A cooling period of 30 minutes preceded the incubation of the slides for HIF-1α with protein block (Novolink Max Polymer detection system, ready to use, Novocastra Laboratories Ltd, Newcastle Upon Tyne, UK) for 5 minutes at room temperature. Incubation of the slides with the HIF-1α mouse monoclonal (BD Biosciences, Pharmingen, Lexington, MA, USA), was done at a dilution of 1:50 overnight at 4°C. For detection, a polymer (Novolink Max Polymer detection system, ready to use) was used. For ER and HER2, the slides were incubated with primary antibodies for ER (1:100, Dako) and HER2 (1:100, Neomarkers) 60 minutes at room temperature.

For PR, Glut-1 and CAIX, antigen retrieval was performed in citrate buffer, pH = 6.0, for 20 minutes at 100°C. A cooling period of 30 minutes preceded the incubation (60 minutes at room temperature) with the primary antibodies. Polyclonal primary antibodies used were: PR (1:100, Dako), Glut-1 (1:200, DAKO) and CAIX (1:1000, Abcam, Cambridge Science Park, Cambridge, UK). For detection of the primary antibodies against ER, PR, HER2, CAIX and Glut-1, a poly HRP anti-Mouse/Rabbit/Rat IgG (ready to use, Immunologic, Duiven, Netherlands) was used. All slides were developed with diaminobenzidine (10 minutes) followed by hematoxylin counterstaining. Before the slides were mounted all sections were dehydrated in alcohol and xylene. Positive controls were used throughout, negative controls were obtained by omission of the primary antibodies from the staining procedure. Representative pictures of positive and negative controls for HIF-1α, CAIX and Glut-1 have been provided as Figure S1.

Scoring of immunohistochemistry was performed by one observer (PJvdD). HIF-1α was regarded overexpressed when >1% of nuclei were positive as described before [26]. ER and PR expression was regarded positive when 10% or more of the tumor nuclei stained positive. HER2 was scored positive when a 3+ membrane staining was observed according to the Dako system. CAIX and Glut-1 stainings were scored positive when a clear membrane staining pattern was seen. Associations between stainings were tested by Chi-square analysis. P-values<0.05 were considered to be statistically significant.

Results

The clinicopathological characteristics and expression of ER, PR, HER2, HIF-1α, CAIX and Glut-1 of BRCA1, BRCA2 and non-BRCA mutation-related DCIS cases are described in Table 1. The age of onset is lower in BRCA1 compared to non-BRCA4 mutation carriers (p = 0.000). BRCA1 mutation-related DCIS cases often are ER, PR and HER2-negative as compared to the BRCA2 and non-BRCA mutation-related DCIS (see Table 1 for correlations).

Expression of hypoxia-induced proteins in BRCA1, BRCA2 and non-BRCA mutation-related DCIS

HIF-1α overexpression was observed in 63% (20/32) of the BRCA1, in 62% (10/16) of the BRCA2 and in 34% (26/77) of the non-BRCA4 mutation-related DCIS cases (p = 0.005;Table 1). CAIX overexpression was observed in 56% (18/32) of BRCA1 mutation-related DCIS cases, with accompanying HIF-1α overexpression in 31% (10/32) of the cases (p = 0.358;Table 2). Glut-1 was overexpressed in 59% (19/32) of the BRCA1 mutation-related DCIS cases and HIF-1α was co-overexpression in 41% (13/32) of these cases (p = 0.403).
CAIX was expressed in 44% (7/16) of BRCA2 mutation-related DCIS cases with accompanying HIF-1α overexpression in 38% (6/16) of the cases (p = 0.091). Glut-1 overexpression was observed in 75% (12/16) of BRCA2 mutation-related DCIS cases, with HIF-1α co-overexpression in 56% (9/16) of the cases (p = 0.074).

In the non-BRCA mutation-related DCIS cases, CAIX expression was seen in 6% (5/77) of the cases which were negative for HIF-1α. Glut-1 was overexpressed in 67% (52/77) of non-BRCA mutation-related DCIS cases, with concomitant HIF-1α overexpression in 29% (22/77) of the cases (p = 0.022).

Furthermore, in the BRCA1 and BRCA2 mutation-related DCIS cases, no correlations between HIF-1α expression and grade, ER, PR and HER2 expression were found. For the non-BRCA mutation-related DCIS cases, a positive trend was observed with grade, and a negative trend with ER (Table 2).

Expression of hypoxia-induced proteins in BRCA1, BRCA2 and non-BRCA mutation-related DCIS and invasive cancer

In the BRCA1 mutation-related cases with DCIS and concomitant invasive cancer (N = 29), the frequency of HIF-1α overexpression was high in both lesions: 62% (18/29) and 83% (24/29), respectively (p = 0.264;Table 3.). The frequency of CAIX expression was 52% (15/29) and 79% (23/29), respectively, in DCIS and invasive carcinoma (p = 0.311). Further, 59% (17/29) of the DCIS and 83% (24/29) (p = 0.945) of the invasive lesions were positive for Glut-1 expression. Examples of these IHC results are shown in Figure 1.

In the BRCA2 mutation-related cases with invasive counterparts (N = 16), 63% (10/16) of DCIS lesions were HIF-1α positive as compared to 38% (6/16) if invasive lesions (p = 0.016). The same expression of CAIX was observed in BRCA2 mutation-related DCIS lesions and the invasive counterpart lesions, 44% (7/16) (Table 1).

### Table 1. Clinicopathological characteristics and expression of ER, PR, HER2, HIF-1α, CAIX and Glut-1 in DCIS lesions of BRCA1, BRCA2 and non-BRCA mutation carriers.

|         | BRCA1 | BRCA2 | non-BRCA | p-value |
|---------|-------|-------|----------|---------|
| N       | 32    | 16    | 77       |         |
| Age     |       |       |          |         |
| <45     | 25(78%) | 9(56%) | 14(18%)  |         |
| >45     | 7(22%) | 7(44%) | 63(82%)  | 0.000   |
| Grade   |       |       |          |         |
| 1       | 0(0%)  | 1(6%)  | 11(14%)  |         |
| 2       | 9(28%) | 8(50%) | 30(39%)  |         |
| 3       | 23(72%) | 7(44%) | 36(47%)  | 0.035   |
| ER      |       |       |          |         |
| neg     | 22(69%) | 4(25%) | 19(25%)  |         |
| pos     | 10(31%) | 12(75%) | 58(75%)  | 0.000   |
| PR      |       |       |          |         |
| neg     | 27(84%) | 9(56%) | 36(47%)  |         |
| pos     | 5(16%) | 7(44%) | 41(53%)  | 0.002   |
| HER2    |       |       |          |         |
| neg     | 31(97%) | 11(69%) | 55(71%)  |         |
| pos     | 1(3%)  | 5(31%) | 22(29%)  | 0.14    |
| HIF-1α  |       |       |          |         |
| neg     | 12(38%) | 6(38%) | 51(66%)  |         |
| pos     | 20(63%) | 10(62%) | 26(34%)  | 0.005   |
| CAIX    |       |       |          |         |
| neg     | 14(44%) | 9(56%) | 72(94%)  |         |
| pos     | 18(56%) | 7(44%) | 56(69%)  | 0.000   |
| Glut-1  |       |       |          |         |
| neg     | 13(41%) | 4(25%) | 25(33%)  |         |
| pos     | 19(59%) | 12(75%) | 52(67%)  | 0.527   |

### Table 2. Correlation of HIF-1α expression in DCIS lesions of BRCA1, BRCA2 and non-BRCA mutation carriers with age, grade, ER, PR, HER2, CAIX and Glut-1 expression in these lesions.

|         | BRCA1 | BRCA2 | non-BRCA | p-value |
|---------|-------|-------|----------|---------|
| N       | 32    | 16    | 77       |         |
| HIF-1α  |       |       |          |         |
| neg     | 8     | 14    | 1        | 0.24    |
| pos     | 4     | 6     | 5        | 0.551   |
| Age     |       |       |          |         |
| <45     | 9     | 16    | 3        | 0.696   |
| >45     | 3     | 4     | 0.740    | 0.427   |
| Grade   |       |       |          |         |
| 1       | 0     | 0     | 1        | 0.14    |
| 2       | 2     | 7     | 4        | 0.081   |
| 3       | 10    | 13    | 21       | 0.045   |
| ER      |       |       |          |         |
| pos     | 4     | 6     | 5        | 0.330   |
| PR      |       |       |          |         |
| pos     | 2     | 3     | 5        | 0.373   |
| HER2    |       |       |          |         |
| pos     | 12    | 19    | 5        | 0.099   |
| CAIX    |       |       |          |         |
| pos     | 4     | 10    | 5        | 0.094   |
| Glut-1  |       |       |          |         |
| pos     | 6     | 13    | 3        | 0.22    |

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Glut-1 was overexpressed in 75% (12/16) of DCIS cases and in 56% (9/16) (p = 0.146) of the invasive BRCA2 mutation-related lesions (Table 3).

The frequency of HIF-1α expression in non-BRCA mutation-related DCIS and concomitant invasive cancer (N = 50) was 38% (19/50) and 34% (17/50), respectively (p = 0.029). Similar CAIX expression was observed in both lesions, 8% (4/50) and 12% (6/50), respectively (p = 0.015). Glut-1 overexpression was seen in 70% (35/50) of DCIS cases and in 36% (18/50) (p = 0.797) of the invasive non-BRCA mutation-related lesions.

In summary, these non-significant differences indicate that HIF-1α positivity was similar in DCIS and the accompanying invasive lesions. Differences in HIF-1α expression between BRCA1 and BRCA2 and non-BRCA mutation related DCIS were borderline significant (p = 0.062). A significant difference in HIF-1α expression was seen between BRCA1 and BRCA2 as compared to non-BRCA mutation-related invasive cancer (p = 0.000).

Expression of hypoxia-induced proteins in BRCA non-BRCA mutation-related DCIS vs invasive cancer

Table 4 shows the expression of HIF-1α, CAIX and Glut-1 in paired, DCIS and concomitant invasive cancer, for BRCA mutation and non-BRCA mutation carriers.

HIF-1α expression was observed in both lesions in 55% (16/29) of the BRCA1 mutation-related cases, whereas both lesions were negative for HIF-1α expression in 10% (3/29) of cases. Overall, in 66% (19/29) of the BRCA1 mutation carrier cases both lesions showed similar expression levels of HIF-1α. In 28% (8/29) of the BRCA1 mutation-related cases only the invasive part, and in 7% (2/29) only the DCIS lesion showed HIF-1α expression. CAIX and Glut1 were expressed in both lesions in 45% (13/29) and 48% (14/29) of the BRCA1 mutation carrier cases, respectively, and both lesions lacked expression of these markers in 14% (4/29) and 7% (2/29) of the cases. Thereby, CAIX was concomitantly expressed in both lesions in 59% (17/29) of the cases, and the Glut-1 in 55% (16/29). Only the invasive lesion of BRCA1 mutation carriers expressed both CAIX and Glut-1 in 34% (10/29) of cases. Expression of CAIX and Glut-1 exclusively in BRCA1 mutation-related DCIS lesions was observed in 7% (2/29) and 10% (3/29) of cases, respectively.

In the BRCA2 mutation-related cases with DCIS and concomitant invasive cancer, 38% (6/16) of the cases HIF-1α expression was observed and was absent in 38% (6/16) of the cases (Table 4). Thus, in 75% (12/16) of the BRCA2 mutation-related cases, the DCIS and invasive lesions of the same patient showed similar expression levels of HIF-1α. Expression of HIF-1α in only the DCIS lesion was seen in 25% (4/16) of the BRCA2 mutation-related cases. CAIX was expressed in both lesions in 31% (5/16) of BRCA2 mutation-related cases and in 44% (7/16) of the cases both lesions lacked expression (total match 75%). CAIX was expressed in the invasive, but not in the DCIS part in 13% (2/16) of the cases, and CAIX was expressed in the DCIS, but not in the invasive part of 13% (2/16) of the cases. Glut-1 was expressed or absent in both lesions in 50% (8/16) and 19% (3/16) of cases, respectively (total match 69%). Further, Glut-1 expression was confined to the invasive part in 6% (1/16) of cases and the DCIS part in 25% (4/16) of the cases.

HIF-1α was expressed in both lesions in 20% (10/50) of the non-BRCA mutation-related cases and both lesions lacked HIF-1α expression in 48% (24/50) of cases. Thus, in total, 68% (34/50) of the non-BRCA mutation carrier cases showed similar expression levels of HIF-1α in both lesions. In 14% (7/50) of the non-BRCA mutation-related cases only the invasive part, and in 18% (9/50) only the DCIS lesion showed HIF-1α expression. CAIX and Glut1 were expressed in both lesions in 4% (2/50) and 26% (13/50), respectively, of the non-BRCA mutation carrier cases. Conversely, both lesions lacked CAIX expression in 84% (42/50) and Glut-1
expression in 20% (10/50) of these cases. Thereby, CAIX expression in both lesions matched in 88% (44/50) and Glut-1 expression in 46% (23/50) of cases. Expression of CAIX and Glut-1 in only the invasive lesion of non-BRCA mutation carriers occurred in 8% (4/50) and 10% (5/50) of cases, respectively, whereas these markers were expressed only in DCIS lesions in 4% (2/50) and 44% (22/50) of cases.

When BRCA1 and BRCA2 mutation-related cases were examined together, HIF-1α expression in DCIS matched the expression in the accompanying invasive cancers in 68% (31/45) of cases, as compared to in 68% (44/50) of non-BRCA mutation carrier cases. The expression of CAIX matched in 64% (29/45) of BRCA1 and BRCA2 mutation-related cases, as compared to in 88% (44/50) of non-BRCA mutation carrier cases. For Glut-1, the expression in DCIS matched the expression in the accompanying invasive cancers in 60% (27/45) of BRCA1 and BRCA2 mutation-related cases as compared to 46% (23/50) for non-BRCA mutation carrier cases.

**Discussion**

Non-BRCA mutation-related DCIS lesions, especially high grade ones, are known to become centrally deprived of oxygen resulting in activation of the hypoxia pathway, as shown in several studies by the presence of HIF-1α and its downstream targets. The aim of the present study was to examine the expression of HIF-1α in DCIS lesions of BRCA1 and BRCA2 mutation carriers in comparison with their invasive counterparts. Activation of HIF-1α in the DCIS stage of BRCA1 or BRCA2 germline mutated patients would indicate that hypoxia is an early driver of BRCA mutation-related carcinogenesis. HIF-1α overexpression was indeed frequently observed in BRCA1 and BRCA2 mutation-related DCIS cases, in association with expression of its downstream genes, indicating that HIF-1α is active.

Overall, 63% (30/48) of BRCA mutation-related DCIS lesions were HIF-1α-positive, which was significantly different compared to non-BRCA mutation carriers (34%, 26/77). The latter figure is

| Table 3. Clinicopathological characteristics and expression of ER, PR, HER2, HIF-1α, CAIX and Glut-1 in DCIS and accompanying invasive lesions of BRCA1, BRCA2 and non-BRCA mutation carriers. |

| BRCA1 | Invasive | DCIS | neg | pos | p-value | neg | pos | p-value | neg | pos | p-value |
|-------|---------|------|-----|-----|---------|-----|-----|---------|-----|-----|---------|
| HIF-1alpha | | | | | | | | | | | |
| DCIS | 38 | 41 | 0.21 | 24 | 19 | 0.003 |
| BRCA2 | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 60 | 72 | 0.016 | 2 | 5 | 0.049 |
| non-BRCA | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 24 | 7 | 0.029 | 2 | 2 | 0.0015 |

**Table 4. Correlation of HIF-1α, CAIX and Glut-1 between invasive and DCIS lesions of BRCA1, BRCA2 and non-BRCA mutation carriers.**

| BRCA1 | Invasive | HIF-1alpha | CAIX | Glut-1 | neg | pos | p-value | neg | pos | p-value | neg | pos | p-value |
|-------|---------|------------|------|-------|-----|-----|---------|-----|-----|---------|-----|-----|---------|
| DCIS | 3 | 8 | 0.264 | 2 | 13 | 0.311 |
| BRCA2 | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 6 | 0 | 0.016 | 2 | 5 | 0.049 |
| non-BRCA | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 24 | 7 | 0.029 | 2 | 2 | 0.0015 |

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| Table 4. Correlation of HIF-1α, CAIX and Glut-1 between invasive and DCIS lesions of BRCA1, BRCA2 and non-BRCA mutation carriers. |

| BRCA1 | Invasive | HIF-1alpha | CAIX | Glut-1 | neg | pos | p-value | neg | pos | p-value | neg | pos | p-value |
|-------|---------|------------|------|-------|-----|-----|---------|-----|-----|---------|-----|-----|---------|
| DCIS | 38 | 41 | 0.21 | 24 | 19 | 0.003 |
| BRCA2 | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 60 | 72 | 0.016 | 2 | 5 | 0.049 |
| non-BRCA | | | | | | | | | | | |
| Invasive | HIF-1alpha | | | | | | | | | | |
| DCIS | 24 | 7 | 0.029 | 2 | 2 | 0.0015 |

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lower compared to our earlier observations where 67% of sporadic DCIS lesions were HIF-1α positive [22]. Nevertheless, the current study suggests that hypoxia and HIF-1α already play a similar role in the DCIS stage of BRCA2 mutation-related carcinogenesis as in non-BRCA2 mutation-related DCIS. We conclude that BRCA1 and BRCA2 germline mutation-related DCIS show a high frequency of overexpression of HIF-1α and its downstream proteins CAIX and Glut-1, as compared to non-BRCA2 mutation-related DCIS. This suggests that hypoxia may already play a role at the DCIS stage of BRCA1 and BRCA2 germline mutation-related breast carcinogenesis, and may also drive cancer progression. The current findings could be clinically relevant for BRCA2 mutation-related breast cancer treatment in several ways. First, HIF-1α and its downstream effectors may be used as molecular imaging targets for early detection and monitoring of therapy response. Second, HIF-1α is an interesting therapeutic target at the pre-invasive stage of BRCA2 mutation-related breast disease to prevent invasive disease.

Supporting Information

Figure S1  Positive controls: Immunohistochemical staining of HIF-1α and CAIX in renal clear cell carcinoma (B and D) and for Glut-1 in placental tissue (F). In A, C and E the primary antibody was omitted to provide negative controls. (TIF)

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Author Contributions

Conceived and designed the experiments: PvdG PJvD EFvW. Performed the experiments: PvdG YHCMS EvdW. Analyzed the data: PvdG PJvD YHCMS EFvW. Contributed reagents/materials/analysis tools: PvdG PJvD YHCMS MEGEMM RBvBIL FHM JB EvdW. Wrote the paper: PvdG PJvD MEGEMM RBvBIL FHM EGdW EFvW.

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