Hypoxia-inducible factor-1α expression and breast cancer recurrence in a Danish population-based case control study

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Abstract

Background: Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that facilitates the adaptation of cancer cells to hypoxic conditions and may be prognostic of breast cancer recurrence. We evaluated the association of HIF-1α expression with breast cancer recurrence, and its association with timing of breast cancer recurrence.

Methods: In this population-based case-control study, we included women diagnosed with stage I–III breast cancer between 1985 and 2001, aged 35–69 years, registered in the Danish Breast Cancer Group. We identified 541 cases of breast cancer recurrence among women with estrogen receptor (ER)-positive disease who were treated with tamoxifen for at least 1 year (ER+TAM+). We also enrolled 300 breast cancer recurrence cases among women with ER-negative disease, not treated with tamoxifen, who survived at least 1 year (ER−/TAM−). Controls were recurrence-free breast cancer patients at the time of case diagnosis, matched to recurrence cases on ER/TAM status, date of surgery, menopausal status, cancer stage, and county of residence. Expression of HIF-1α was measured by immunohistochemistry on tissue microarrays. We fitted logistic regression models to compute odds ratios (ORs) and 95% confidence intervals (CIs) associating HIF-1α expression with recurrence, and with timing of recurrence.

Results: HIF-1α expression was observed in 23% of cases and 20% of controls in the ER+/TAM+ stratum, and in 47% of cases and 48% of controls in the ER−/TAM− stratum. We observed a near-null association between HIF-1α expression in both ER/TAM groups (ER+/TAM+ OR = 1.21, 95%CI 0.88, 1.67 and ER−/TAM− OR = 0.97, 95%CI 0.68, 1.39). HIF-1α expression was not associated with time to recurrence among women in the ER+/TAM+ stratum, but was associated with early recurrence among women in the ER−/TAM− stratum.

Conclusion: In this study, HIF-1α expression was not associated with breast cancer recurrence overall but may be associated with early recurrence among women diagnosed with ER− breast cancer.

Keywords: Breast cancer recurrence, Hypoxia-inducible factor 1, Tamoxifen resistance, Prognostic marker

Introduction

Nearly 90% of women diagnosed with breast cancer survive more than 10 years after their diagnosis [1]. Although targeted treatment protocols have contributed to improved survival, approximately 20–40% of breast cancer patients will have a recurrence by 20 years after their initial diagnosis [2, 3]. This substantial and
prolonged risk of recurrence contributes to distress among breast cancer survivors [4]. Currently, the primary prognostic indicators for late recurrence include lymph node status and stage of breast cancer, which also predict early recurrence [2]. Novel biomarkers are needed to improve identification of patients who are at high risk of late recurrence, allowing for risk stratification of patients who may benefit from more intensive follow-up or prolonged treatment.

As tumors or metastases grow, cells in the tumor’s interior are more distant from blood supplies, leading to a hypoxic tumor microenvironment [5]. Tumor cells need to adapt to this hypoxic environment to facilitate tumor progression [6]. Hypoxia-inducible factor 1 (HIF-1α) is a transcription factor that facilitates the adaptation of cancer cells to hypoxic conditions [7], and may therefore serve as a prognostic marker for late recurrence [8]. Previous studies have shown that hypoxia-induced signaling enables tumor cells to survive during metabolic stress and to enter a prolonged quiescent state of tumor dormancy [9]. Additionally, a number of HIF target genes affect angiogenesis and proliferation of tumor cells, and the emergence from tumor dormancy to proliferative growth [10, 11]. HIF-1α expression is not detected in normal breast tissue, but is present in breast tumors, supporting its potential for use as a prognostic marker for cancer recurrence [7].

In this study, we evaluated the association between HIF-1α expression and breast cancer recurrence and its association with timing of breast cancer recurrence. In a set of exploratory analyses, we described differences in HIF-1α expression between primary and paired recurrent breast tumors, and evaluated if conservation of HIF-1α expression between primary and recurrent tumors was also associated with late recurrence.

Materials and methods
Study population
The study population and data collection have been described in detail elsewhere [12]. Briefly, the source population included stage I–III Danish female breast cancer patients, ages 35–69 years, diagnosed between 1985 and 2001, and registered with the Danish Breast Cancer Group (DBCG) [13]. Since 1977, the DBCG has enrolled nearly all Danish breast cancer patients younger than 70 years at diagnosis into its clinical database. Eligible patients were divided into two strata. The first stratum included patients whose tumors expressed estrogen receptor (ER) (≥ 10% of cells) and who were treated with tamoxifen (TAM) for at least one year (ER+/TAM+, n = 1826 patients). The second stratum included patients whose tumors did not express ER, who were not treated with TAM, and who survived at least one year (ER−/TAM−, n = 1808 patients). Patients not meeting these criteria were excluded (n = 7617 patients). Stratifying by ER and TAM status allowed separation of HIF-1α as predictive of TAM resistance, in which case an association would be observed in only the ER+/TAM+ stratum, and as a prognostic marker, in which case an association would be observed in both strata. Follow-up time was calculated from one year after breast cancer surgery until the first of (a) breast cancer recurrence, (b) death from any cause, (c) loss to follow-up, (d) completion of 10-year of follow-up, or (e) September 1, 2006.

Cases were defined as women with a diagnosis of a local, regional, or distant recurrence, or a contralateral breast cancer registered in the DBCG during follow-up [14]. Controls were selected from members of the source population who were not diagnosed with breast cancer recurrence nor with contralateral breast cancer at the follow-up time of the matched cases’ recurrence. Controls were matched to cases on ER/TAM group, menopausal status at diagnosis, date of breast cancer surgery (caliper matched+/− 12 months), county of residence, and Union for International Cancer Control (UICC) cancer stage at diagnosis. In the ER+/TAM+ stratum, 541 cases were identified; all were included in the analysis. In the ER−/TAM− stratum, 300 cases were identified, and frequency matched according to the distribution of stage and calendar period of diagnosis among the ER+/TAM+ case patients. Given the study design, the case-control odds ratio (OR) provides an unbiased estimate of the rate ratio for the association between HIF-1α expression and breast cancer recurrence rate [15].

Data and tumor tissue collection
Every Danish citizen or legal resident is assigned a unique 10-digit Civil Personal Registration (CPR) number which allows unambiguous linkage across Danish registries [16]. We used the DBCG registry to obtain the following information: demographics (age, menopausal status, county of residence at diagnosis, and treating hospital), tumor characteristics (size, histology, histologic and nuclear grade, nodal involvement, ER status, and TNM stage), surgery type (mastectomy or breast conserving), radiation therapy, and receipt of chemotherapy and TAM therapy.

Tissue microarray construction and immunohistochemistry
In Denmark, all paraffin blocks from pathological specimens are routinely archived after diagnosis. Patient CPR numbers were used to link patients in the study population to the Danish Pathology Data Bank, enabling us to locate and retrieve tumor blocks for 85% of study subjects [17]. For each case and control, formalin-fixed, paraffin-embedded (FFPE) primary tumor tissue
blocks were retrieved from the pathology archives of treating hospitals. Paired FFPE blocks were collected when available for the local and distant recurrences of the 841 cases of breast cancer recurrence in the study sample. The purpose of collecting the recurrent tissues was to assay HIF-1α expression in the recurrent tumor, with the goal of comparing its expression at primary diagnosis with its expression at recurrence diagnosis. Laboratory personnel were blinded to all clinical information including case or control status, ER status, and receipt of TAM therapy.

Tissue microarrays (TMA) were constructed for the primary tumors \( (n = 1434, 85\%) \) as well as the recurrent tumors \( (n = 269, 32\%) \). Figure 1 illustrates the selection of study subjects to be used in the analysis. TMAs were constructed using standard techniques. A fresh section was cut from each study participant’s paraffin block and stained with hematoxylin and eosin. The diagnosis was confirmed by a study pathologist. Areas containing invasive breast carcinoma were identified and marked. Core samples (1 mm diameter) were subsequently removed from each tumor donor block and re-embedded in a
new recipient paraffin TMA block using a TMA Master
Arrayer (3DHISTECH, Budapest, Hungary). If sufficient
material was available, representative tumor \( (n=3) \) and
marginal tissue \( (n=1) \) cores were sampled. Liver and placent-
cal cores were included in each TMA to facilitate ori-
entation within the TMA during microscopy.

**HIF-1α assay**

Immunohistochemistry (IHC) stains were performed on
3 µm TMA tissue sections according to standard proto-
cols. Slides were stained using the Envision Flex+ sys-
tem (Agilent). Slides went through deparaffinization and
epitope retrieval in a PT link with a low pH buffer
(PT Link, Agilent, Santa Clara, CA 95051, USA). Next,
epitope retrieval staining was carried out on an Auto-
stainer Link 48 (Agilent). Endogenous enzyme activity
was blocked for 10 min using EnVision FLEX Peroxidase-
Blocking Reagent (Agilent, SM801). Sections were then
incubated overnight 4 °C with the primary antibody
HIF-1-α (clone EP1215Y) (abcam ab51608) in a 1:1000
dilution, followed by FLEX HRP secondary for 30 min
(Agilent, SM802) and diaminobenzidine chromogene
(DAB) (Agilent: EnVision FLEX DAB+ Chromogen
(DM827) and EnVision FLEX Substrate Buffer (SM803))
for 10 min. Slides were counterstained using Hema-
toxylin (Agilent, EnVision FLEX Hematoxylin (K8008))
for 8 min. Slides were then mounted and scanned on a
Hamamatsu Nanozoomer 2.0HT.

**TMA core scoring**

Expression of HIF-1α was quantified with an H-score
that incorporated staining intensity and percentage of
positively stained tumor cells [18]. Staining intensity was
a weighted scale ranging from 0 for no staining to 3 for
high intensity staining. Percent positivity ranged from 0
to 100% based on percentage of positively stained tumor
cells. In a set of sensitivity analyses, we used percent pos-
itiveness to quantify HIF-1α expression.

Two authors (LJC and SHD) developed a rubric for
intensity levels and a scoring schematic of cytoplasmic
HIF-1α expression (Fig. 2). HIF-1α levels in the cyto-
plasm are not transcriptionally active but are translo-
cated to the nucleus where they exert biological activity.
Levels of HIF-1α in the cytoplasm increase with response
to hypoxia and therefore represent cancer cell adaptation
to a hypoxic tumor microenvironment. Subsequently,
all study cores were rated by one evaluator (LJC), who
was blinded to patient characteristics and case–control
status at the time of scoring. Cores that could not be
scored were excluded, either because the core section
on the TMA was absent or inadequately represented,
or because of poor staining of the core, and this exclu-
sion was addressed in the analysis. An average of cores
available was used to assign a value of HIF-1α expression
based on the average H-score and percent positivity for
each patient.

**Analytic variables**

**Expression of HIF-1α**

The exposure of interest for this study was cytoplasmic
expression of HIF-1α, which we defined as positive ver-
sus negative (H-score > 10 as positive expression versus
H-score ≤ 10 as negative expression). This cutpoint was
based on the 90th quantile of the distribution of the aver-
age H-score of HIF-1α expression among the controls
(Additional file 1: Figure S1A and S1B). Among the cases,
expression of HIF-1α in the paired recurrent tumors
was compared with HIF-1α expression in the primary
tumor. The difference in average H-score in the recurrent
tumor and the average H-score in the primary tumor was
used to determine if there was a decrease (difference in
H-scores ≤ -5), increase (difference in H-scores ≥ 5), or
no change in HIF-1α expression (difference in H-scores
between -5 and 5).

**Breast cancer recurrence**

The study followed the DBCG definition of breast can-
cer recurrence, i.e. any contralateral or ipsilateral breast
cancer occurring locally, regionally, or distally, after
breast cancer diagnosis. As noted above, all recurrences
occurred one to ten years after initial breast cancer diag-
nosis. Time to recurrence was categorized by approxi-
mate quintiles among cases: 1 to < 2 years; 2 to < 3 years; 3
to < 4 years; 4 to < 6 years; and 6 to 10 years.

**Covariates**

We included UICC stage (I, II, III), grade (I, II, III), meno-
pausal status at diagnosis (premenopausal/postmeno-
pausal), receipt of chemotherapy (yes/no), receipt of
radiotherapy (yes/no), surgery type (mastectomy/breast
conserving surgery), year of diagnosis, age at diagnosis,
and county of residence in each analysis. During the study
enrolment period, guidelines for TAM duration changed
from one year to two years, and finally to five years of
adjuvant therapy. Therefore, to account for the progres-
sion of duration in the guidelines, we adjusted for TAM
treatment duration (years) in the ER+/TAM+ stratum.

**Statistical analysis**

Analyses were stratified by the ER/TAM grouping to
evaluate whether HIF-1α expression was predictive of
tamoxifen resistance, prognostic of breast cancer recurrences, or neither predictive nor prognostic. We
first report the covariate distributions as frequency
and percent by case and control status within ER/TAM
group. We additionally present descriptive statistics of
ER/TAM group, HIF-1α expression in primary tumor, and time to recurrence by change in HIF-1α expression (decrease, no change, increase), comparing HIF-1α expression in the recurrent versus primary tumor.

In the conventional analysis, we used logistic regression to estimate the association between HIF-1α expression and breast cancer recurrence. To avoid discarding matched sets due to missing tumor core samples, we used unconditional multivariable logistic regression adjusting for the matched factors and other covariates to compute the ORs and 95% confidence intervals (CIs) reflecting the association of HIF-1α expression with recurrence.

To estimate the association between HIF-1α expression and time to recurrence, we calculated five ORs and the 95% CIs within the approximate quintiles of time to recurrence, adjusting for the same covariates included in the conventional analysis. We then regressed the natural logarithm of the ORs (lnOR) on time to recurrence (represented by \( i = 5 \) midpoints), weighting with the inverse variance of lnOR. The beta-estimate from this approach reflects the association between HIF-1α expression and time to recurrence.

In an exploratory analysis, we examined the role of conservation of HIF-1α expression—defined as positive expression in both the primary and recurrent tumor—and late recurrence. We calculated the OR associating conservation of HIF-1α expression with late recurrence.
With positive HIF-1α expression in the primary tumor. Among cases with recurrent tissue available, 61% of cases had an increase, and 28% had a decrease in HIF-1α expression. Women in the ER+/TAM+ stratum were more likely to have a decrease in HIF-1α expression compared with those without HIF-1α expression (OR = 1.21, 95% CI: 0.88, 1.67) (Table 3). Similarly, in the ER+/TAM− stratum, we observed a near-null association between HIF-1α expression and breast cancer recurrence (OR = 0.97, 95% CI 0.68, 1.39). Accounting for potential selection bias with IPPW due to missing tumor cores yielded little change in the estimates of association across both ER/TAM groups.

In Table 4 we report the association between HIF-1α expression and time to recurrence. Across both ER+/TAM+ and ER+/TAM− strata, HIF-1α expression was associated with recurrence in years 3 to < 4 (OR = 3.41, 95% CI 1.28, 9.06 and OR = 2.50, 95% CI 0.81, 7.68, respectively), although the estimates were imprecise. However, no association was observed in other categories of time to recurrence, and there was no discernable pattern in the association between HIF-1α expression and breast cancer recurrence by category of time to recurrence. When we regressed the lnOR on time to recurrence, we observed no association in the ER+/TAM+ group (β = 0.005, 95% CI −0.23, 0.24), but a negative association in the ER+/TAM− group (β = −0.27, 95% CI −0.62, 0.08), suggesting that HIF-1α expression was associated with early recurrence in the ER+/TAM− group (Table 5).

In our exploratory analysis of the association between conservation of HIF-1α expression—positive HIF-1α expression in primary and recurrent tumors—and late recurrence, we observed an OR = 4.32 (95% CI 0.92, 20) for the association between conservation of HIF-1α expression and late breast cancer recurrence compared with loss of HIF-1α expression in the recurrent tumor in the ER+/TAM+ group. We were unable to calculate a reliable OR in the ER+/TAM− as there was only 1 case that had positive expression in both the primary and recurrent tumors, and a late recurrence.

The sensitivity analyses, which assessed HIF-1α positivity based on percent positive of tumor cells, yielded similar results to the analyses assessing HIF-1α expression based on the H-score (Additional file 1: Tables S2–S6).

Discussion
In this study, we did not observe an association between HIF-1α expression and breast cancer recurrence or timing of breast cancer recurrence among women in the ER+/TAM+ stratum. However, we observed that HIF-1α expression may be associated with early recurrence among women in the ER+/TAM− stratum.

Previous studies have reported mixed associations between HIF-1α expression and breast cancer prognosis.
A recent meta-analysis, which included 14 studies, reported that high HIF-1α expression among breast cancer patients was an indicator of poor prognosis, and was associated with both overall survival (hazard ratio [HR] = 1.46, 95%CI 1.12, 1.92) and disease-free survival (HR = 1.91, 95%CI 1.43, 2.57) [21]. However, studies included in the meta-analysis were relatively small (<750 patients) and were heterogeneous with respect to positive HIF-1α expression classification. Dales et al. reported that overexpression of HIF-1α was associated with early...
Table 2  Change in HIF-1α expression between primary tumor and recurrent tumor by ER/TAM group and time to recurrence among 269 recurrences from the ProBe CaRe population-based case control study

| Characteristics                  | Change in HIF-1α expression |        |        |        |
|----------------------------------|-----------------------------|--------|--------|--------|
|                                  | Decrease                    | No change | Increase |
|                                  | Total                       |        |        |        |
|                                  | n   | %  | n   | %  | n   | %  |
| Total                            | 76 | (28) | 130 | (48) | 63 | (23) |
| Median change [IQR]              | −18.2 [−44.2, −8.0]        | 0 [−1.3, 1.3] | 21.3 [9.5, 54] |
| ER/TAM group                     |    |       |     |       |对他    |       |
| ER+/TAM+                         | 41 | (25) | 84 | (51) | 38 | (23) |
| ER−/TAM−                         | 35 | (33) | 46 | (43) | 25 | (24) |
| HIF-1α category                  |    |       |     |       |对他    |       |
| Positive                         | 63 | (79) | 2  | (2.5) | 15 | (19) |
| Negative                         | 13 | (6.9)| 128| (68) | 48 | (25) |
| Time to recurrence, years        |    |       |     |       |对他    |       |
| 1 to < 2                         | 30 | (31) | 43 | (45) | 23 | (24) |
| 2 to < 3                         | 12 | (21) | 32 | (56) | 13 | (23) |
| 3 to < 4                         | 7  | (16) | 26 | (58) | 12 | (27) |
| 4 to < 6                         | 17 | (37) | 18 | (39) | 11 | (24) |
| 6–10                             | 10 | (40) | 11 | (44) | 4  | (16) |

ER, estrogen receptor; HIF-1α, hypoxia-inducible factor 1; IQR, interquartile range; TAM, tamoxifen

Table 3  Association between HIF-1α expression and breast cancer recurrence by ER/TAM group among 1682 subjects from the ProBe CaRe population-based case control study

| HIF-1α expression | ER+/TAM+ breast cancer patients | ER−/TAM− breast cancer patients |
|-------------------|---------------------------------|---------------------------------|
|                   | Recurrent cases/ controls | Adj. OR (95% CI)a | IPPW OR (95%CI) | Recurrent cases/ controls | Adj. OR (95% CI)a | IPPW OR (95%CI) |
| Positive          | 106/93                      | 1.21 (0.88, 1.67) | 1.19 (0.87, 1.63) | 122/122                     | 0.97 (0.68, 1.39) | 0.91 (0.60, 1.36) |
| Negative          | 352/371                     | Reference | Reference | 136/132                     | Reference | Reference |

Adj, adjusted; CI, confidence interval; ER, estrogen receptor; HIF-1α, hypoxia-inducible factor 1; IPPW, inverse probability of participant weighting; OR, odds ratio; TAM, tamoxifen

a Adjusted for matching factors (menopausal status, surgery date, county of residence, stage, age category) and chemotherapy, radiation therapy, and tamoxifen duration (ER+ stratum only)

Table 4  Association between HIF-1α expression and breast cancer recurrence by quintile of recurrence time and ER/TAM group among 1682 subjects from the ProBe CaRe population-based case control study

| Time to recurrence | Median time to recurrence (years) | ER+/TAM+ breast cancer patients | ER−/TAM− breast cancer patients |
|--------------------|----------------------------------|---------------------------------|---------------------------------|
|                    | Cases/controls | Adjusted OR (95% CI)a | Cases/controls | Adjusted OR (95% CI)a |
| 1 to < 2           | 1.5            | 109/113 | 1.03 (0.54, 1.96) | 113/105 | 0.73 (0.42, 1.27) |
| 2 to < 3           | 2.4            | 80/85  | 1.16 (0.55, 2.44) | 67/72  | 0.74 (0.36, 1.54) |
| 3 to < 4           | 3.4            | 88/78  | 3.41 (1.28, 9.06) | 32/33  | 2.50 (0.81, 7.68) |
| 4 to < 6           | 4.7            | 113/115| 0.79 (0.41, 1.52) | 27/27  | 0.76 (0.22, 2.60) |
| 6–10               | 7.3            | 68/73  | 1.39 (0.59, 3.30) | 19/17  | 5.78 (0.61, 55.0) |

CI, confidence interval; ER, estrogen receptor; OR, odds ratio; TAM, tamoxifen; y, years

a Adjusted for matching factors (menopausal status, surgery date, county of residence, stage, age category) and chemotherapy, radiation therapy, and tamoxifen duration (ER+ stratum only)
recurrence among breast cancer patients, but ER status was not recorded for study participants [8]. The results reported by Dales et al. are consistent with our results among ER−/TAM− breast cancer patients. Another study examined the association between HIF-1α expression and recurrence-free survival in a cohort of premenopausal breast cancer patients from a randomized trial of TAM therapy [22]. The authors concluded that HIF-1α expression was associated with recurrence among those who did not receive TAM (HR = 1.4, 95% CI 0.9, 2.3).

Tumor hypoxia is an adaptive mechanism by which tumor cells are able to survive in the oxygen deprived tumor microenvironment, supporting cancer cell progression [5]. HIF-1α is frequently activated in tumors, which decreases hypoxia-induced apoptosis and increases stress-induced proliferation of solid tumors [23]. Some studies have reported that ER expression is inversely associated with HIF-1α expression [7, 24]. These reports are consistent with those observed in the current study, as women in the ER−/TAM− group were more likely to have positive HIF-1α expression. Moreover, we observed that HIF-1α expression was associated with an early time to recurrence among those in the ER−/TAM− group. Our exploratory analyses suggested that breast cancer patients in the ER−/TAM− group who had positive HIF-1α expression in their primary and recurrent tumors were more likely to have had early recurrence (<5 years), although the small sample size yielded imprecise results.

A limitation of this study is that it was restricted to breast cancer diagnoses between 1985 and 2001. During this period, TAM represented guideline-concordant care for postmenopausal women, and for premenopausal women beginning in 1999, but is now frontline adjuvant hormone therapy only for premenopausal women. However, postmenopausal women for whom aromatase inhibitors are contraindicated or poorly tolerated still receive TAM; so our results are still applicable to that target population [25]. Although screening protocols were being developed during this time period, Denmark did not implement a nationwide breast cancer screening program until 2007, after this study’s enrollment period [26]. An additional concern is that the study population consisted primarily of women initially diagnosed with stage II (48%) and stage III (48%) disease, largely due to DBCG criteria for TAM therapy during the study’s diagnostic period [27]. However, TAM has for some time been prescribed to women with stage I ER+ breast cancer as guideline-concordant therapy. We were unable to assess recurrence risk after 10 years of follow-up, as the DBCG follows breast cancer patients for a recurrence for up to 10 years. Recurrences that occur after 10 years may be of interest for future studies. It is possible that we imperfectly measured HIF-1α expression in this study. We used the average of 1–3 cores per patient to mitigate any variation in scoring or intratumor heterogeneity and evaluated HIF-1α expression using both an average H-score and average percent positivity with consistent results. Finally, we only had recurrent tumors for a subset of the recurrent cases (32%). This is expected as women diagnosed with a distant recurrence are unlikely to have primary surgery as part of their care, but it limited our ability to estimate the association between changes in HIF-1α expression and timing of recurrence.

Conclusions
In conclusion, in this population-based case–control study, we found no evidence of an association between HIF-1α expression and breast cancer recurrence, or timing of recurrence among ER+/TAM+ postmenopausal breast cancer patients. We observed some evidence of an association between HIF-1α expression and early recurrence among ER−/TAM− breast cancer patients. Future studies may be strengthened by examination of HIF-1α expression in premenopausal breast cancer patients and conservation of HIF-1α expression in tumors over time.

Abbreviations
HIF-1α: Hypoxia-inducible factor-1α; ER: Estrogen receptor; TAM: Tamoxifen; OR: Odds ratio; CI: Confidence interval; DBCG: Danish Breast Cancer Group; UICC: Union for International Cancer Control; CPR: Civil Personal Registration; FFPE: Formalin-fixed, paraffin-embedded; TMA: Tissue microarray; IHC: Immunohistochemistry; IPPW: Inverse probability of participant weighting; HR: Hazard ratio.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1186/s13058-021-01480-1.

Additional file 1: Supplementary materials for distribution of HIF-1α, availability of tumor cores, and analyses using percent positivity of HIF-1α.

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Authors’ contributions
LJC, MLM, DCF, TLL conceptualized the research question; LIC, MLM, TLL, DCF, TPA designed the methodologic approach; HTS, DCF, SHD, TLL secured funding support and facilitated data acquisition; KBC, SHD, RY, LIC constructed the tissue microarrays, performed the immunohistochemistry staining, developed

**Table 5** Association between HIF-1α expression with time to recurrence by ER/TAM group among 1682 subjects from the ProBe CaRe population-based case control study

| ER/TAM Group | Intercept (SE) | Effect estimate β (SE) | 95% CI  |
|--------------|---------------|------------------------|--------|
| ER+/TAM+     | 0.18 (0.50)   | 0.005 (0.12)           | (−0.23, 0.24) |
| ER−/TAM−     | 0.95 (0.51)   | −0.27 (0.18)           | (−0.62, 0.08) |

CI, confidence interval; ER, estrogen receptor; SE, standard error; TAM, tamoxifen
the scoring rubric, and scored the tumour cores; LC, LCM, SPM performed the analyses; LJC, LLM, DCF, TPA, PD, PMC, HTS contributed to interpretation of study results; LJC drafted the initial manuscript, all authors provided comments and edits. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available upon request to the corresponding author and the Department of Clinical Epidemiology. The data are not publicly available due to privacy and ethical considerations.

Declarations

Ethics approval and consent to participate
The study was approved by the Danish Data Protection Agency, the Danish Ethical Committee, and the Emory University Institutional Review Board.

Consent for publication
Not applicable.

Competing interests
The authors have no conflict of interest to declare.

Novelty and impact
Hypoxia-inducible factor-1α (HIF-1α) facilitates adaption of cancer cells to hypoxic tumor microenvironments and may be prognostic of breast cancer recurrence. In this study, we evaluated the association of HIF-1α expression with breast cancer recurrence and timing of recurrence in a population-based case-control study. We observed a near-null association between HIF-1α expression and breast cancer recurrence overall, and that HIF-1α expression was not associated with early recurrence among women diagnosed with estrogen receptor-negative breast cancer.

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