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ORIGINAL ARTICLE – CANCER RESEARCH

Prognostic impact of PIK3CA protein expression in triple negative breast cancer and its subtypes

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Abstract

Background Triple negative breast cancer (TNBC) harbors a heterogeneous group of carcinomas with poor prognosis and high genetic variability. As a potential aim for targeted therapy, genetic mutations leading to an activation of the phosphoinositide 3-kinase pathway in a catalytic subunit (PIK3CA) in breast cancer have been analyzed currently. Little is known about the clinical impact and prognostic or predictive value of this marker in TNBC subtypes.

Methods Samples from 119 TNBC cases were submitted to immunohistochemical PIK3CA protein expression analysis and scored semi-quantitatively as negative, weak (1+), or strongly expressed (2+). Expression scores were correlated to patient's characteristics, imaging features, and TNBC subtypes. TNBC subtypes were categorized into four subtypes: basal like, mesenchymal like, luminal androgen receptor (LAR), and immunomodulatory.

Results We did not observe differences in clinical aspects and imaging features between TNBC with and without PIK3CA expression. PIK3CA expression was in general higher in the LAR subtype. The disease-free survival and overall survival were significantly better in TNBC with PIK3CA protein expression, independent of TNBC subtypes.

Conclusion Despite conflicting results in the literature, our study clearly shows a better outcome of PIK3CA-expressing TNBC, independent of TNBC subtypes. PIK3CA expression in TNBC is not associated with specific clinical or diagnostic features. Further molecular studies and meta-analysis are warranted to clarify the prognostic and predictive role of PIK3CA protein expression.

Keywords Triple negative breast cancer · PIK3CA protein expression · PI3K pathway · Imaging features · Breast cancer subtypes

Background

Triple negative breast cancer (TNBC) is a highly diverse group of cancers and is defined by the lack of both estrogen receptor (ER) and progesterone receptor (PR) expression, and of human epidermal growth factor receptor 2 (HER2) amplification (Dent et al. 2007). TNBC is associated with advanced disease stage and worse prognosis due to high proliferation and aggressive behavior, and accounts for 10–20% of breast cancers (Kaplan and Malmgren 2008). As it harbors a highly variable group of breast cancers, categorization into molecular subtypes was recently established (Lehmann et al. 2011; Turner and Reis-Filho 2013). Carcinogenic mutations and marker expressions in TNBC are avidly followed in the literature with the aim of improving therapy options. The focus is on cellular pathways that can be addressed by targeted therapies (Pop et al. 2018). One of the most promising approaches is the phosphoinositide

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3-kinase (PI3 K) and serine/threonine kinase AKT pathway, regulating cellular functions such as proliferation, cell survival, and differentiation (Engelman et al. 2006). Activating mutations in PIK3CA, which encodes the α-catalytic subunit of PI3K, play an important role in carcinogenesis and cancer progression not only in the breast, but also in colorectal, endometrial, and other cancers (Meyer et al. 2011; Meyer et al. 2011). PIK3CA protein expression in breast cancer is described in various frequencies and can be observed in up to 45% of cases (Pop et al. 2018; Meyer et al. 2011; Koboldt et al. 2012). Alteration of the PI3K pathway is associated with worse outcomes of targeted therapies in Her2-positive breast cancer (Meyer et al. 2011). Furthermore, it correlates with cancer cell resistance to endocrine therapies of hormone receptor-positive breast cancer (HR +) causing a poorer outcome (Miller et al. 2011). In contrast, PIK3CA expression is more frequent in Luminal A tumors that are known to have a better prognosis (Koboldt et al. 2012).

However, the prognostic role of PIK3cA protein expression, especially in TNBC, is still controversial (López-Knowles et al. 2010; Cizkova et al. 2012). To date, little is known about the clinical features of TNBC and its subtypes expressing PIK3CA. As higher tumor stage worsens the prognosis of TNBC, initial diagnosis and early treatment are crucial (Deniz et al. 2019). A clinical relevance would be obvious if marker expression had an impact on early diagnosis and treatment. This should also be taken into account when interpreting outcome data of TNBC expressing PIK3CA.

To advance the understanding of diagnostic and potential therapeutic usage, we observed PIK3CA expression within TNBC subtypes, imaging features, clinical features, outcomes, and characteristics of 119 patients with TNBC. Tumors were categorized into four subtypes: basal like (BL), mesenchymal like (ML), luminal androgen receptor (LAR), and immunomodulatory (IM).

Materials and methods

Patients and clinical data

In total, data of 166 patients diagnosed with TNBC between 2002 and 2016 in the Breast Center Zurich, Switzerland, were collected. To create a subgroup which represents the current standard in terms of diagnosis and therapy, we excluded 47 patients (28.3%) from analysis. The main criterion was the application of chemotherapy regimen with anthracycline and taxane, currently considered to be standard in TNBC (Wahba and El-Hadaad 2015). Overall, 34 patients (20.5%) did not receive standard chemotherapy. Furthermore, patients with smaller tumors (< 1 cm) ($n = 4$; 2.4%), and with incomplete diagnostic information ($n = 9$; 5.4%) were excluded from the analysis of clinical data, diagnosis, and outcome. The patients' medical records were reviewed for personal data, clinical symptoms, diagnostic process and treatment. The median follow-up was 60 months (95% CI 50–72 months, inverse Kaplan–Meier method).

TNBC subtypes

Surgical specimens and/or core biopsies of patients with TNBC underwent histological review regarding subtyping. Specimens were processed as formalin-fixed, paraffin-embedded tumor tissues according to the standardized protocol of the institute. TNBC was defined as lacking the expression of ER and PR (< 1% by immunohistochemistry), and for Her2 (assessed by immunohistochemistry and/or by fluorescence in situ hybridization). In cases of pathologic complete response (pCR; $n = 8$), initial biopsy specimen was submitted for subtyping. Immunostains were performed in the Laboratory of Special Technics in the Department of Pathology and Molecular Pathology, University Hospital Zurich, using standardized procedures, ready-to-use antibodies and the automatic Benchmark staining machines.

From the surgical specimen, we constructed a tissue microarray (TMA) and few preoperative core biopsies (at pCR) were used as whole sections for the analysis. TMA construction was conducted as described previously as is summarized in the publication from Kündig et al. (2018). Invasive carcinomas of the primary tumor were identified on HE sections. Two cores of 0.6 mm each were punched from each area and transferred to an empty paraffin block under the guidance of a precision tool. Molecular subtyping was performed using a panel of antibodies based on the recommendation of Lehmann et al. (2011), and Turner and Reis-Filho (2013). Subtyping was done immunohistochemically using a semi-quantitative scoring system from 0, +, and +++.

Immunophenotyping

The subtyping of the TNBC cases was done under consideration of the criteria of Lehmann et al. (2011) and Turner and Reis-Filho (2013) as follows: LAR subtype was defined as apocrine morphology on HE stains along with PIK3CA, CK5/6 and AR expression. ML subtype was defined as metaplastic morphology on HE stains along with claudin 1 (low) and CK5/6 expression. BL subtype was defined as any non-special subtype on HE along with CK5/6 expression. IM subtype was defined as any subtype with pronounced lymphocytic intratumoral infiltration independently of the marker expression profile.

Immunohistochemical staining

Immunostains were performed using following antibodies and laboratory procedures: PIK3CA: the SIGMA HOA009985 polyclonal antibody on the Ventana autostainer (dilution 1:25 with CC1 standard pretreatment and UltraMap Rabbit signal enhancer). CK5/6: the Ventana D4 antibody on the Ventana autostainer (prediluted with CC + 48 pretreatment and OptiView signal enhancer). Androgen receptors: the Ventana clon F39.4.1 antibody on the Ventana autostainer (dilution 1:50 with P1 pretreatment and OptiView signal enhancer). Claudin 1: the Ventana polyconal antibody on the Ventana autostainer (dilution 1:50 with P1 pretreatment and OptiView signal enhancer). EGFR: the Ventana 31G7 antibody on the Ventana autostainer (dilution 1:50 with P1 pretreatment and OptiView signal enhancer).

Scoring of immunohistochemical stains

PIK3CA: the expressions were scored semi-quantitatively with a three-tiered scoring system (negative, ≤ 50% of the cells positive, > 50% of the cells positive), being cytoplasmic positive. CK5/6: the expressions were scored semi-quantitatively with a three-tiered scoring system (negative, ≤ 50% of the cells positive, > 50% of the cells positive), being cytoplasmic positive. Androgen receptors: the expressions were scored semi-quantitatively with a three-tiered scoring system (negative, ≤ 50% of the cells positive, > 50% of the cells positive), being nuclear positive. Claudin 1: the expressions were scored semi-quantitatively with a three-tiered scoring system (negative, ≤ 50% of the cells positive, > 50% of the cells positive), being cytoplasmic positive. EGFR: the scoring was carried out as recommended by the scoring guidelines: negative (score 0/1 +), score 2 + and score 3 +.

Statistics

For the descriptive analysis, mean (and standard deviation) or median were used for continuous variables and number and percentage were used for categorical variables. To enhance interpretation, stacked bar plots were used to visualize some categorical variables. Chi square and Fisher's exact tests were used to test for differences in proportions across subtypes. Overall survival (OS) and disease-free survival were visualized with Kaplan–Meier plots of survival probability. All the analyses were performed in the R programming language.

Ethics

The study was approved by the local ethics committee of Zurich, Switzerland, according to the national and international ethics guidelines KEK-217-219.

Results

Patient characteristics

Patient characteristics did not show any significant differences between the groups PIK3CA-expressing and non-PIK3CA-expressing tumors (Table 1): Patients' age, reason for first consultation, menopausal status, and family history of breast cancer were equally distributed. Most patients of our study group were primarily treated by surgery ($n = 81$, 68.1%). In 38 patients who were treated with neoadjuvant chemotherapy (NAC), 7 patients (18.4%) showed pCR. Except in one case, all the pCRs were seen in the subgroup without amplification of PIK3CA.

TNBC subtypes

A total of 119 patients with TNBC were included in this study, of whom 47 (39.5%) were categorized into BL subtype, 18 (15.1%) into IM, 24 (20.2%) into ML, and 30 (25.2%) into LAR. PIK3CA expression rate was relatively high, as it was observed in more than every second tumor (59.7%). It was verified more often in the LAR subtype (70% versus 62.5%, 55.3%, and 50.0% in ML, BL, and IM, respectively) (Fig. 1). However, this data does not provide evidence of a significant difference across the four subtypes (Chi square test, $p = 0.48$). H&E morphology was categorized into no special type (NST), and the special types of apocrine, metaplastic, and lymphocytic (Table 1). Metaplastic type was defined as metaplastic morphology on HE stains along with claudin 1 (low) and CK5/6 expression.

Overall survival and disease-free survival did not differ significantly within the TNBC subtypes (p value = 0.29, data not shown).

Clinical imaging and tumor characteristics

BI-RADS classification in mammogram and ultrasound, suspicious lymph nodes and tumor size in clinical imaging, as well as breast density were equally distributed in TNBC with and without expressing PIK3CA. In only seven cases (5.8%), of which five cases were PIK3CA-expressing TNBC, imaging misinterpretation led to significant tumor growth and/or upgrading of tumor state (Table 2). Furthermore, tumor characteristics were similar in both groups regarding tumor size, affected axillary lymph nodes, tumor morphology, and stage IV tumors (Table 1).

Table 1 Patients' and tumor characteristics

	Overall	PIK3CA expression	No PIK3CA expression	*p* value
n	119	71	48	
Age [year; mean (sd)]	54.08 (12.05)	53.28 (11.90)	55.25 (12.29)	0.41
Menopausal status (%)				
Premenopausal	49 (41.2)	30(42.3)	19 (39.6)	0.92
Postmenopausal	70 (58.8)	41 (57.7)	29 (60.4)	
Reason for first consultation (%)				
Self-detected palpable mass	77 (64.7)	46 (64.8)	31 (64.6)	0.75
Screening	34 (28.6)	20 (28.2)	14 (29.2)	
Symptoms (discharge/pain)	4 (3.4)	2 (2.8)	2 (4.2)	
Other	4 (3.4)	3 (4.2)	1 (2.1)	
Family history of breast cancer (%)				
Yes	25 (21.0)	13 (18.3)	12 (25.0)	0.52
No	94 (79.0)	58 (81.7)	36 (75.0)	
Histopathologic tumor size (%)				
ypT0	7 (5.9)	1 (1.4)	6 (12.5)	0.12
ypT1	20 (16.8)	12 (16.9)	8 (16.7)	
ypT2-4	11 (9.2)	5 (7.0)	6 (12.5)	
pT1	35 (29.4)	19 (26.8)	16 (33.3)	0.11
pT2-4	46 (38.7)	34 (47.9)	12 (25.0)	
Histopathologic axillary lymph node (%)				
ypN0	14 (11.8)	9 (12.7)	5 (10.4)	0.29
ypN1-3	18 (15.1)	7 (9.9)	11 (22.9)	
pN0	58 (48.7)	33 (46.5)	25 (52.1)	0.14
pN1-3	29 (24.4)	22 (31.0)	7 (14.6)	
HE morphology (%)				
Any special type	32 (26.9)	17 (23.9)	15 (31.2)	0.50
NST	87 (73.1)	54 (76.1)	33 (68.8)	
Tumor grading (%)				
Higher differentiated (G1 and G2)	17 (14.3)	9 (12.7)	8 (16.7)	0.73
G1	1 (0.8)	0	1 (2.0)	
G2	16 (13.4)	9 (12.7)	7 (14.6)	
Poor differentiated (G3)	102 (85.7)	62 (87.3)	40 (83.3)	
Primary distant metastasis (%)				
Yes	3 (2.5)	1 (1.4)	2 (4.2)	0.56
No	116 (97.5)	70 (98.6)	46 (95.8)	

Outcome

We compared the final survival outcomes for patients with TNBC expressing or non-expressing PIK3CA. The disease-free survival of patients with PIK3CA expression in the tumor tissue was greater than for patients not expressing this marker (*p* value 0.011; Fig. 2). The overall survival curve for the group with PIK3CA expression also remained significantly higher than for the group of non-expressing PIK3CA (log rank test, *p* value 0.037; Fig. 3).

We also analyzed overall survival and disease-free survival in the two subgroups of BL and non-BL TNBC subtypes, as well as metaplastic and non-metaplastic TNBC. In the groups of BL and non-BL TNBC, no significant difference was observed (additional material, Fig. 6). TNBC with metaplastic morphology were few in numbers ($n=9$; 7.6%), and two-thirds of these showed a PIK3CA expression ($n=6$; 66.7%; Figs. 4 and 5). Interestingly, all patients with a metaplastic morphology of TNBC had a distinctly better outcome (additional material, Fig. 7).

Discussion

Activating mutations of the PIK3CA/PTEN pathway leading to PIK3CA protein expression are frequent mainly in the luminal subtype of breast cancer, whereas the incidence of PIK3CA expression in TNBC differs within the literature

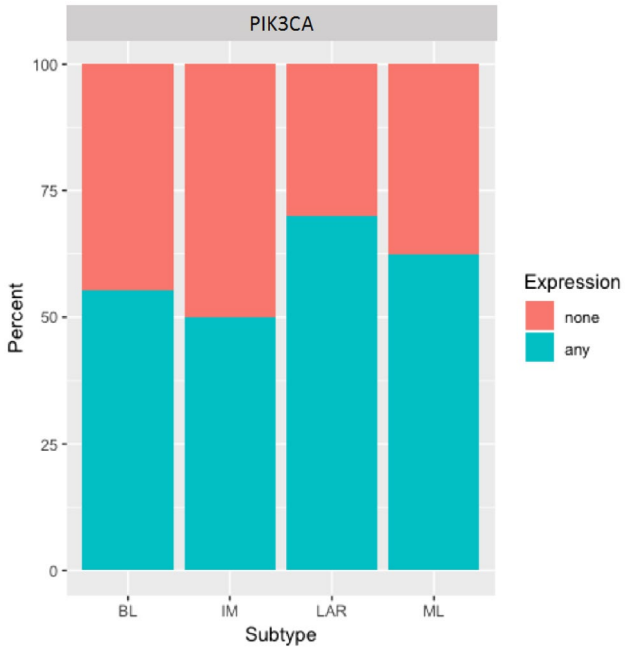

Fig. 1 PIK3CA expression by subtype (binary distribution as negative or positive independently from the expression score of weak or strong)

(Koboldt et al. 2012; Kündig et al. 2018; Shah et al. 2012). There is evidence to suggest that there is great potential for targeting therapy in PIK3CA expressing tumors, but as yet little data exist about clinical and prognostic factors of PIK3CA in TNBC (Anderson et al. 2016). In a recent study by Jiang et al., the genomic landscape of TNBC subtypes showed a higher incidence of PIK3CA expression in the LAR subtype, emphasizing different genetic signatures in TNBC subtypes (Jiang et al. 2019).

Somatic PIK3CA protein expression

Several PIK3CA mutations have been described in literature so far, most of which leads to a PIK3CA protein expression, but non-sense variants are also known (Uscanga-Perales et al. 2019). Considering clinical aspects, we analyzed TNBC subtypes with PIK3CA expression independently from the inducing mutation. It should be noted that if some activating mutations presented a different clinical impact than others, this would not be clarified by our study. This needs to be considered in light of the fact that the presented approach focuses on the final common pathway and can be easily reproduced using routine pathological methods.

Histopathologic analysis

In most clinical studies, PIK3CA analyses were performed in biopsy specimens before NAC, which may risk the underestimation of PIK3CA expression frequency in heterogeneous tumors. Our study group included 31 surgical specimens (26.1%) of patients who received NAC vs. 81 specimens of patients with primarily surgical treatment. PIK3CA expression frequency did not differ within these groups. A recent study showed no increase in somatic expression after NAC , which supports our results (Kim et al. 2018). Only in a small group of patients with pCR after NAC ($n=7$), pre-therapeutical biopsy specimen were analyzed and showed an underrepresentation of PIK3CA-expressing TNBC ($n=1$; 1.4% of the PIK3CA versus $n=6$; 12.5% of the non-PIK3CA). Generally, pCR was associated with better outcome, especially in TNBC (Cortazar et al. 2014), which might be in contrast to a better overall outcome of our marker-expressing TNBC subgroup. However, TNBC has a high genomic instability, and PIK3CA expression in the pre-therapeutic biopsy specimen might differ from the surgical specimen. As numbers of the pCR subgroup is low in our study, interpretation is limited.

TNBC subtypes

The presented study is to our best knowledge the first which focuses on clinical and imaging features of the PIK3CA protein expression in the four TNBC subtypes. Subtyping was performed in accordance to Turner and Reis-Filho (2013), with three main molecular/gene expression profiles: basal-like cancers (BL), mesenchymal-like cancers (ML), and luminal androgen receptor cancers (LAR) (Turner and Reis-Filho 2013). A further subtype of immunomodulatory cancers (IM) is defined by a substantial lymphocytic infiltration. Overall, PIK3CA was expressed in 59% ($n=98$) of all TNBC, which is concordant to recently published literature, however, TNBC with far less PIK3CA expression have also been described (Koboldt et al. 2012). The highest expression rate was found in LAR (70.2% versus 62.5%, 55.3%, and 50.0% in ML, BL, and IM, respectively), which is consistent with earlier studies (Jiang et al. 2019; Lehmann et al. 2014). Despite our results not being statistically significant, the trend clearly supports the data in the current literature and implies a potential benefit of cell-cycle inhibitors such as CDK4/6 inhibitors in LAR-TNBC tumors.

Morphologically, metaplastic TNBC is defined by various combinations of poorly differentiated components and is associated with poor prognosis and lower response to neoadjuvant chemotherapy (Rayson et al. 1999). However, discrimination of metaplastic tumors is meaningful and molecular similarities between the mesenchymal subtype of TNBC and metaplastic histology are obvious (Basho et al. 2018). Metaplastic TNBC show a tendency for better OS after treatment with mTOR inhibition than non-metaplastic TNBC (Basho et al. 2018), and they more often present PIK3CA expression than TNBC of no special type (Ng et al. 2017;

Table 2 Clinical imaging features of TNBC expressing PIK3CA and non-expressing PIK3CA

	Overall	PIK3CA expression	No PIK3CA expression	*p* value
n	119	71	48	
Tumor size in ultrasound in mm [median (IQR)]	20 (14, 28)	21 (14, 30)	20 (14, 25)	0.33
Tumor size in mammogram in mm [median (IQR)]	20 (12, 25)	20 (12, 27)	20 (11, 25)	0.88
Unifocal tumor (%)				
Yes	100 (84)	61 (85.9)	39 (81.2)	0.67
No	19 (16)	10 (14.1)	9 (18.8)	
BI-RADS mammography (%)				
Benign features (1–3)	32 (26.9)	19 (26.8)	13 (27.1)	1.00
Malignant features (4–5)	87 (73.1)	52 (73.2)	35 (72.9)	
Breast density (%)				
Low (ACR a and b)	51 (42.9)	30 (42.3)	21 (43.8)	1.00
High (ACR c and d)	68 (57.1)	41 (57.7)	27 (56.2)	
BI-RADS ultrasound (%)				
Benign features (1–3)	15 (12.6)	9 (12.7)	6 (12.5)	1.00
Malignant features (4–5)	104 (87.4)	62 (87.3)	42 (87.5)	
Sonographic suspicious lymph nodes (%)				
Yes	47 (39.5)	30 (42.3)	17 (35.4)	0.58
No	72 (60.5)	41 (57.7)	31 (64.6)	
Patients with benign features in ultrasound (*n*)	15	9	6	0.98
Diagnostic delay (month) [mean (sd)]	1.7 (2.4)	2.3 (3.0)	0.8 (1.0)	
Patients with benign features in mammogram (*n*)	32	19	13	
Diagnostic delay (month) [mean (sd)]	1.0 (2.0)	1.2 (2.4)	0.7 (1.5)	0.98
Tumor growth during delay (%)				
< 1 cm	2 (1.7)	0 (0)	2 (4.2)	0.38
≥ 1 cm	3 (2.5)	2 (2.8)	1 (2.1)	
≥ 1 cm and newly diagnosed lymph node metastasis	4 (3.4)	3 (4.2)	1 (2.1)	

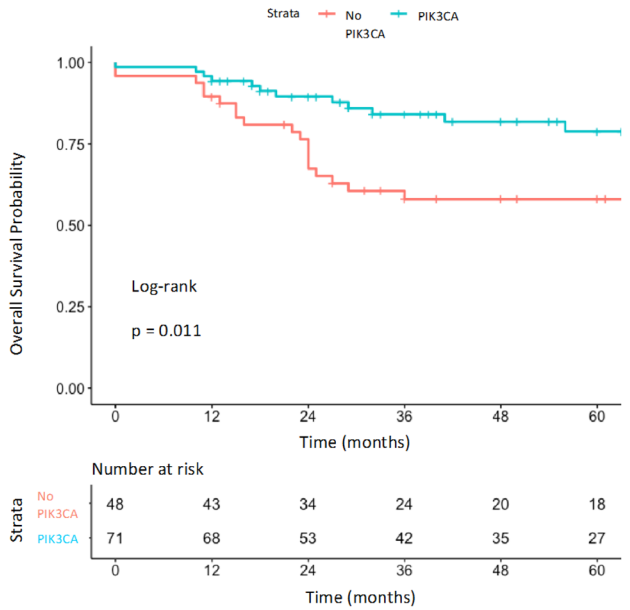

Fig. 2 Kaplan–Meier curves of disease-free survival for patients with and without PIK3CA expression

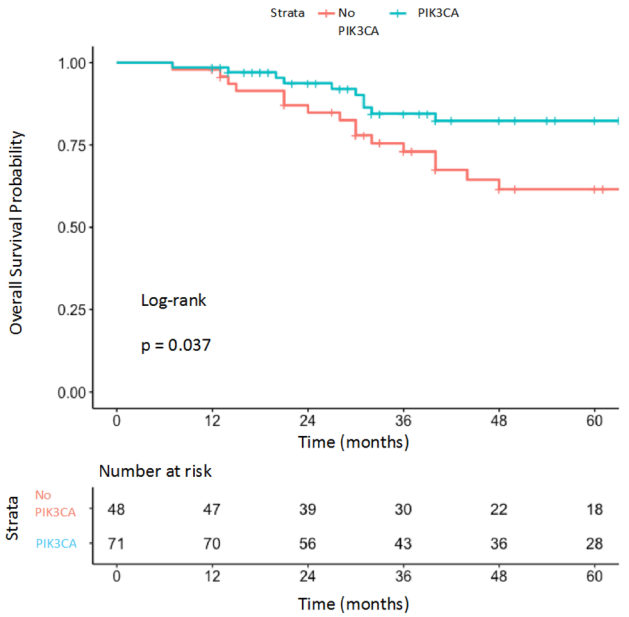

Fig. 3 Kaplan–Meier curves of overall survival for patients with and without PIK3CA expression

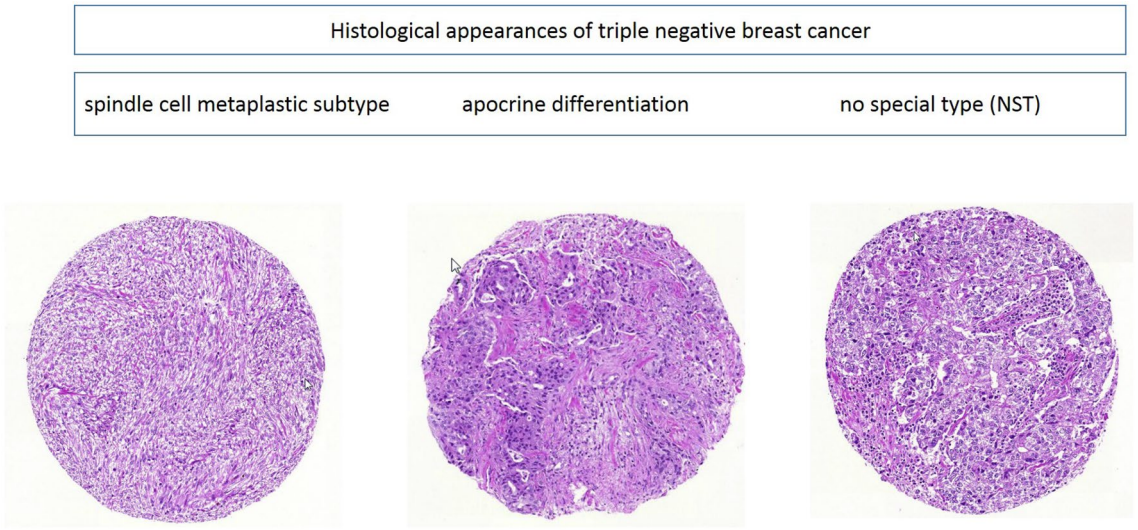

Fig. 4 Histological appearances of triple negative breast cancer. HE (hematoxylin and eosin) stain

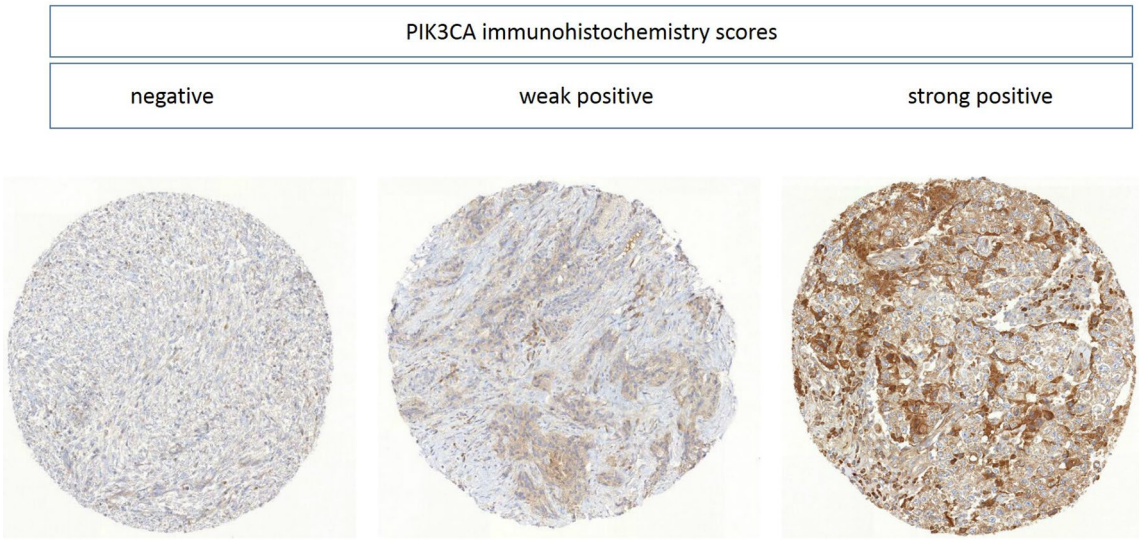

Fig. 5 PIK3CA immunohistochemistry scores (negative, weak positive, strong positive)

Hennessy et al. 2009). In a study by Bartels et al. (2018), the majority of 34 patients with the special type of MCB in TNBC showed PIK3CA protein expression. In our study, 6 out of 9 (66.7%) carcinomas with metaplastic histology expressed PIK3CA. Metaplastic morphology was classified on HE stains as described in the current WHO classification for breast tumors as TNBC harboring squamous and/or spindle cell neoplastic tumor components (Lakhani et al. 2012). All metaplastic carcinomas in our series corresponded to these two morphological subtypes. Interestingly, OS of all metaplastic TNBC was clearly better than in non-metaplastic TNBC, which corresponds to recently published data (Basho et al. 2018). However, these results need to be interpreted with caution because of the limited number of metaplastic tumors in our study group.

Patients' and tumor characteristics

Imaging features of PIK3CA-expressing TNBC and nonexpressers showed no difference in sonographic and mammographic characteristics. Malignant signs such as spiculated mass, microcalcification, and suspicious lymph nodes did not differ among the groups. The number of tumors with benign imaging features, that may lead to misinterpretation and diagnostic delay, is also similar—around 12% in ultrasound, and 27% in mammogram. These data imply that the

clear prognostic value of PIK3CA expression is not influenced by different tumor size or tumor stage at diagnosis, lymph node characteristics in ultrasound, nor misinterpretation of imaging findings. Few publications have addressed the clinical characteristics of PIK3CA-expressing TNBC so far. PIK3CA expression in HER2-positive breast cancer was associated with higher pathologic lymph node category compared to the wild type but did not affect pCR and OS (Seo et al. 2018). In our study, the pathological results after surgery did not differ within the groups of expressing and non-expressing PIK3CA. Tumor size, lymph node status, grading, and metastasis were equally distributed, and can therefore be excluded as potential bias of the outcome analysis. This also applies to patients' characteristics such as age, menopausal status, reason for first consultation, and breast cancer family history.

Outcome

There have been conflicting outcome results in patients with PIK3CA expression in TNBC, but frequency also shows big differences in the current literature (López-Knowles et al. 2010; Chen et al. 2019; Santarpia et al. 2012). Our study shows a significantly better disease-free and overall survival rate in the long-term follow-up of patients with PIK3CA-expressing TNBC. These results are consistent with a study by Kalinsky et al. (2009), showing an improved outcome in both TNBC and hormone receptor-positive BC with PIK3CA expression. As normal breast tissue can also harbor PIK3CA-expressing cells, a possible protective role in the carcinogenesis of highly aggressive tumors with poor prognosis needs to be considered (Myers et al. 2016).

Outcome of our study group did not differ within BL and non-BL TNBC, despite a better response to NAC in BL TNBC being previously reported (Lehmann et al. 2011). In a subgroup of patients with metaplastic morphology of TNBC ($n=9$, of which 6 cases showed a PIK3CA expression), outcome was clearly better than in TNBC of no special type. By small numbers, this result has to be interpreted with caution. However, it may indicate more efficiency for systemic taxane- and antracylin-based chemotherapy. Regarding the outcome of TNBC subtypes independently from PIK3CA expression, there was no significant difference in our study group.

Limitations

Our study has some limitations that need to be considered. Above all, this was a retrospective analysis of patients who were diagnosed over a 14-year period. On the other hand, we provided a comprehensive data selection to create a homogeneous study group which is comparable to the current situation in which we included only those patients who received taxane- and anthracycline-based chemotherapy regimens. In doing so, we excluded from the entire cohort ($n=166$) 34 patients (20.5%) who for various reasons (older patients deemed unfit for more aggressive chemotherapy regimens and/or patient's non-compliance) received only mono-chemotherapy regimens. This process of data selection is highly relevant in interpreting outcome results of TNBC, and earlier publications often did not consider these biases (Pop et al. 2018; Bonotto et al. 2014).

To validate PIK3CA as a reliable prognostic—and probably predictive—marker, further studies with larger numbers are warranted.

Conclusion

Clinical features and imaging characteristics do not differ within the group of PIK3CA expression and non-expression in TNBC. Molecular subtyping of TNBC shows a slightly higher percentage of PIK3CA expression in the LAR subtype, and in HE morphology a higher percentage in metaplastic TNBC. Our results suggest that PIK3CA protein expression is clearly associated with improved overall survival and disease-free survival in TNBC, independent of TNBC subtypes.

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Author contributions All authors made substantial contributions to the study and they have approved the current version and agreed to publication.

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Data availability The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was conducted following the protocol approved by the Cantonal Ethics Committee of Zurich (BASEC-Nr. 2017-00219) and in accordance with Good Clinical Practice Guidelines.

Informed consent Informed consent was obtained from all individual participants included in the study.

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