Chasing Hippos: Implications of YAP1 and TAZ Expression in Pregnancy-Associated Breast Cancer Tumorigenesis

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Abstract

Background: Hippo signaling pathway de-regulation has been strongly associated with tumorigenesis, metastasis, and drug resistance. Pregnancy-associated breast cancer, the most frequently diagnosed malignancy during gestation, demonstrates an intricate molecular nature. The aim of the present study is to evaluate Hippo pathway transducers TAZ and YAP1 expression in pregnancy-associated breast cancer in relation to the clinicopathological characteristics of the disease.

Methods: Formalin-fixed paraffin-embedded tissues from 21 PABC patients treated at Alexandra Hospital in Athens, Greece were immunohistochemically analyzed. Statistical analysis was performed to investigate a possible correlation among the Hippo pathway signaling and the clinical features of the disease.

Results: In 48% of patients included in the study strong nuclear TAZ/YAP1 staining in tumor cells was identified. Additionally, the hormone receptor negative status was statistically correlated with strong positivity of the TAZ/YAP1 co-transcriptional factors. No association was observed with the overall-survival and the disease-free survival rate.

Conclusions: Hippo pathway is proven to be de-regulated in a subset of pregnancy-associated breast cancer patients highlighting the complex molecular background of the disease which certainly requires further investigation.

Introduction

The Hippo signaling pathway was firstly identified in genetic studies of *Drosophila melanogaster* and was classified as a major suppressor of tissue overgrowth [1, 2]. Since then, numerous studies on human cells have been conducted providing valuable insight into the intricate role of Hippo pathway in tumorigenesis. As it is well established, a well-conserved kinase cascade comprising Hippo pathway maintains tissue homeostasis by regulating cell proliferation and apoptosis [3, 4]. More particularly, the main components of the Hippo signaling MST1/2 (mammalian Ste20-like kinase 1/2) phosphorylate thus activating LATS1/2 (large tumor suppressor 1/2). These core kinases further phosphorylate two transcriptional coactivators named YAP1 (yes-associated protein 1) and TAZ (transcriptional coactivator with PDZ-binding motif), which are then retained in the cytoplasm through binding to 14-3-3 protein and are ultimately digested through ubiquitination and proteasomal degradation [4, 5]. When the abovementioned pathway is switched off though, the Hippo key transducers TAZ and YAP1 translocate to the nucleus combining with the TEAD (transcriptional enhanced associated domain) transcription factors 1–4, thus demonstrating significant oncogenic function [5, 6]. Multiple theories have evolved regarding Hippo signaling de-regulation and cross talking with several pathways (e.g., Wnt/ β -catenin, TGF- β , JNK) leading to carcinogenesis, metastasis, and drug resistance [7, 8]. Additionally, the Hippo signaling pathway has been extensively explored in various tumors including metastatic breast cancer, triple-negative breast cancer (TNBC), and male breast cancer [7, 9, 10]. To the best of our knowledge this is the

first study evaluating YAP1 and TAZ expression in pregnancy-associated breast cancer (PABC), a truly challenging situation with significantly increasing incidence rate.

PABC is generally defined as breast cancer diagnosed anytime during gestation, lactation or within one year after delivery [11]. Every year 1 in 3,000–10,000 women is diagnosed with PABC which represents the most frequently diagnosed pregnancy-related malignancy [12]. As women tend to defer childbearing to a later age, PABC incidence is expected to increase substantially in the upcoming years. Additionally, the application of non-invasive prenatal testing (NIPT) in all pregnant women aiming to detect fetal abnormalities, has unquestionably increased the identification of asymptomatic PABC patients [13, 14]. The genomic profile of PABC has recently been addressed in a systematic review published, but the underlying mechanisms of the disease still require further investigation in order to explain PABC aggressiveness; advanced T stage in diagnosis, nodal involvement, high histologic grade, negative estrogen receptor (ER) and progesterone receptor (PR) status and HER-2 overexpression [15, 16].

Toward this effort, the study of Hippo pathway transducers TAZ and YAP1 in PABC aims to further elucidate the oncogenic mechanism of PABC and to detect signal molecules which may serve as novel diagnostic biomarkers and therapeutic targets of anticancer drugs.

Materials And Methods

This cohort study of patients diagnosed with PABC was conducted at the Alexandra Hospital that is affiliated with the National and Kapodistrian University of Athens in Greece. All participants were required to sign the informed consent form according to the principles of the Declaration of Helsinki, to have completed the 18th year of age and to have attended the Breast Unit of the Department of Obstetrics and Gynecology or the Department of Clinical Therapeutics at Alexandra Hospital in Athens, Greece. The study protocol was approved by the Institutional Review Board (IRB) of Alexandra Hospital.

Participants

By the time the written informed consent was granted, all medical records of women diagnosed with PABC in our Institution according to the inclusion criteria were retrospectively reviewed for the period 2000–2019. Additionally, all women diagnosed with the disease from January 2020 until December 2020 were also prospectively included in our study. The following clinicopathological data regarding PABC, and the patients' demographic characteristics were extracted from the medical files of each eligible patient; date of birth, gestational age at diagnosis, family cancer history, histopathologic evaluation (tumor size, stage, grade, lymph node status, hormone receptor and HER-2 expression), genetic testing, PABC treatment (e.g., surgery, chemotherapy, immunotherapy, hormonotherapy, radiotherapy), and follow-up data. Furthermore, for each patient enrolled in the study a sample of formalin-fixed paraffin-embedded (FFPE) tissue was obtained from the Department of Pathology in Alexandra Hospital to evaluate Hippo pathway transducers expression.

Immunohistochemistry

TAZ and YAP1 transducers expression in PABC was detected on FFPE tissue using the rabbit anti-YAP and anti-TAZ antibodies, clone D24E4 of the ImmPress REAGENT KIT, UNIVERSAL, Anti-MOUSE/RABBIT Ig, VECTOR, at the dilution 1:50 and according to the manufacturer’s protocol. Their expression was reported both in terms of percentage of tumor-expressing cells and staining intensity (0 = absent, 1 + = weak, 2 + = moderate, 3 + = strong). The topographic TAZ and YAP1 expression was evaluated in both the cytoplasm and the nucleus of the tumor cells, and in the main cellular components, namely endothelial cells, non-lymphocytic stromal cells, and tumor-infiltrating lymphocytes (TILS), that were morphologically identified [9]. Nuclear staining, which demonstrated variable expression, was further classified as negative/weak positive or strong positive following the study by Rodríguez-Núñez et al.; tissue was given a score which resulted from multiplying the nuclear staining intensity from 0 (no staining) to 3 (strong staining) by the extension based on the percentage of positive cells (from 0–3). Thus, samples were grouped as negative or weak positive (scores 0–2), and strong positive (scores 3–9) [17].

Statistical Analysis

Descriptive statistics were used to summarize study participants’ characteristics. The Pearson’s Chi-Squared Test and the Fisher Exact Test, when appropriate, for categorical variables were used to investigate the correlation among the immunohistochemical YAP1/TAZ expression and the clinicopathological characteristics. Survival curves were estimated with the Kaplan-Meier method. All the statistical analyses were performed using the SPSS v24 software. *p*-value < 0.05 was considered statistically significant.

Results

Patient characteristics

Overall, 21 women diagnosed with PABC were enrolled in this study and were screened for the expression of the Hippo signaling transducers TAZ and YAP1. Detailed data regarding the demographic variables and the pregnancy characteristics of the patients are demonstrated in Table 1 including age, gestational age at diagnosis, ethnicity, and family cancer history. The age at diagnosis ranged from 28 to 42 years, with a mean age of 35.1 years (SD: 3.83; range: 28.0-42.0). Six patients (38%) were diagnosed with PABC within the first year after delivery, whereas 10 patients (63%) were diagnosed during pregnancy. Most of the participants reported a negative family cancer history (76%), whereas only 5 patients (24%) had a positive family history of lung, prostate, breast, or colorectal cancer among first degree relatives.

Table 1: Patient characteristics

Age (y) at diagnosis		
Mean $\pm$ SD	35.05 $\pm$ 3.39	
Median (min-max)	36.00 (28-42)	
Gestational age at diagnosis	N	N (%)
1 st trimester	1	6%
2 nd trimester	2	13%
3 rd trimester	7	44%
Postpartum	6	38%
N/A	5	24%
Ethnicity		
Greek	18	86%
non-Greek	3	14%
Family cancer history		
Positive	5	24%
Negative	16	76%

No association was observed between YAP1/TAZ nuclear expression, the gestational age at diagnosis, and the family cancer history.

Histopathological characteristics

The histopathological features of the 21 PABC patients examined in this study are summarized in Table 2. The most frequently diagnosed histopathological type was invasive ductal carcinoma (IDC) (81%), whereas a few cases of invasive lobular (ILC) and metaplastic carcinoma were also identified. The vast majority of tumors was of high grade (81%) and the mean value of tumor size was 4.21cm (SD: 3.01; range: 0.8-12.0). In most cases analyzed, high levels of Ki-67 > 20% were detected (75%). Only 2 women (10%) were diagnosed with primary metastatic PABC. Nineteen patients received adjuvant chemotherapy (90%). The majority had a diagnosis of disease stage II (52.4%).

Table 2: Histopathological characteristics

Characteristics		N (%)
Histopathological Type	Invasive ductal carcinoma (IDC)	81
	Invasive lobular carcinoma (ILC)	9.5
	Metaplastic carcinoma	9.5
Tumor Grade	Grade II	19
	Grade III	81
Tumor Size	4.21 cm (0.8-12)	
Hormone Receptor (HR) Status	Positive	60
	Negative	40
Estrogen Receptor (ER) Status	Positive	50
	Negative	50
Progesterone Receptor (PR) Status	Positive	50
	Negative	50
HER-2	Positive	45
	Negative	55
Ki-67	> 20%	75
	< 20%	25
Stage	I	14.3
	II	52.3
	III	23.9
	IV	9.5
Molecular Subtypes	Luminal A	14.3
	Luminal B / HER-2 positive	28.6
	Luminal B / HER-2 negative	14.3
	TNBC	23.8
	HER-2 positive	14.3
	N/A	4.8

The nuclear YAP1/TAZ staining was classified as negative/weak positive in 52% (11/21) and as strong positive in 48% (10/21) of patients analyzed. As far as HRs' expression is concerned, HR negative status was associated with strong nuclear expression of the Hippo pathway transducers TAZ and YAP1 in tumor cells (p -value = 0.006). Furthermore, when evaluating separately the ER and the PR expression, a statistically significant correlation was identified solely among the PR negative status and the strong positive nuclear YAP1/TAZ expression (p -value = 0.007). Representative patterns of TAZ and YAP1 immunohistochemical staining are illustrated in Figure 1. No significant association was observed with the histopathological type, the tumor grade and size, the HER-2 and the Ki-67 expression, the molecular subtype (Luminal A, Luminal B - HER-2 positive/negative, TNBC, HER-2 positive) and the stage of the disease.

YAP1/TAZ nuclear expression and prognosis

We next investigated the impact of the Hippo signaling TAZ and YAP1 expression on the overall survival (OS) and the disease-free survival (DFS) rate of PABC patients. Follow-up data were retrieved from the medical files of 15 out of 21 PABC patients enrolled in this study; 2 deaths were recorded, and the remaining 13 patients demonstrated a mean OS rate of 172.348 months (Figure 2A, 95% CI: 134.305 - 210.392). Furthermore, as far as progression-free disease is concerned the following events were reported; local breast relapse was observed in four patients, liver and bone metastases developed in three patients, lung metastasis in two, and brain metastasis in a single patient. In total, 6 patients were characterized as metastatic; 2 primary metastatic PABC patients, one of which showed further signs of relapse with bone and lung metastases 5 months after the initial diagnosis, and 4 secondary metastatic patients. The progression-free survival (PFS) rate was not estimated as the cohort was too small to draw any conclusions. Additionally, the mean DFS rate was estimated to be 86.453 months (Figure 2B, 95% CI: 54.283 – 118.622). Even though more patients characterized by strong positive nuclear YAP1/TAZ staining relapsed or passed away, no statistically significant correlation was identified with the OS and the DFS rate.

Discussion

PABC is the most common malignancy accompanying pregnancy [16]. The distinct clinical and genetic data of PABC are not fully elucidated [15]. Herein we have conducted a translational study of Hippo pathway transducers with the clinicopathological characteristics of PABC. To our knowledge, we are the first to publish data connecting Hippo pathway with PABC.

Hippo pathway was found to be de-regulated in 10/21 cases (48%), implying that almost half of PABC patients may present with this abnormality in their transcriptional programs. When we further studied the correlation between HR status and Hippo pathway activity, we observed a statistically significant result for HR negative patients and YAP1/TAZ strong nuclear positivity (p = 0.006). This result was also prominent for PR negative cases (p = 0.007). These findings are in accordance with previous publications showing that PABC are commonly tumors with low HR status [16, 18]. Additionally, our data confer a

possibly crucial molecular mechanism for the development of a subset of PABC, which generally are shown to have complex genetic and molecular background [8, 15].

When we tested the histopathological type, the tumor grade and size, the HER-2 status and the Ki-67 expression, the molecular subtype, and the stage of the disease, we did not find any statistically significant results. Possibly these findings depict the molecular complexity of the disease [15, 19–21]. Hippo signaling seems not to be present and meaningfully active in all PABC cases. Furthermore, Hippo signaling positivity was not correlated with statistically significant results with the gestational age at diagnosis of PABC during or after pregnancy. Here, it should be highlighted that our data show a higher percentage of patients being diagnosed during pregnancy than postpartum, as is published by others [16].

Our study did not reveal any correlations between OS, DFS and Hippo pathway. Even though, more patients with Hippo pathway de-regulation experienced relapse or died from the disease, there were no statistically significant results. However, longer follow up may be needed to extract more accurate conclusions for these clinical parameters.

The characteristics of our study population are representative of PABC patients [16, 18]. The majority harbor the aggressiveness of the disease with high Ki-67 levels, low HR status and grade 3 tumors. Only 2 (9.5%) patients were primarily metastatic, 4 developed metastases at a later course of their disease, while 4 patients experienced local relapse. Stage II and III patients were the majority in agreement with previous publications. Furthermore, luminal B and TNBC patients were most commonly found in our cohort, also in agreement with previous publications [16].

The present study has several limitations that need to be addressed. Meaningful information was not reported in 6 cases, thus decreasing the number of our patients with clinical data at 15 included cases and as a result, the statistical power was limited and anticipated associations were not observed. Even though our study is limited by the retrospective nature of the analysis and its small number of patients, it adds an important amount of clinical data regarding the clinical characteristics and outcome of PABC, as well as the significance of Hippo pathway in the development of this rare disease.

Conclusions

To conclude, Hippo pathway signaling is de-regulated in a subset of PABC patients. We are the first to report that almost half of PABC patients had strong nuclear immune-positivity for YAP1/TAZ co-transcription factors, implying high activity and de-regulation of Hippo pathway. HR negative tumors were statistically associated with Hippo pathway depicting an important role of this molecular signaling in the development of these PABC tumors. PABC remains a rare tumor entity with distinct clinical and molecular characteristics, with Hippo pathway offering a crucial component of the molecular complexity of these tumors.

Abbreviations

PABC: pregnancy-associated breast cancer; MST1/2: mammalian Ste20-like kinase 1/2; LATS1/2: large tumor suppressor 1/2; YAP1: yes-associated protein1; TAZ: transcriptional coactivator with PDZ-binding motif; TEAD: transcriptional enhanced associated domain; TNBC: triple-negative breast cancer; NIPT: non-invasive prenatal testing; ER: estrogen receptor; PR: progesterone receptor; HR: hormone receptor; PFS: progression-free survival; DFS: disease-free survival; OS: overall-survival.

Declarations

Ethics approval and consent to participate

The present study was performed in accordance with the ethical standards of the Institution and followed the tenets of the Helsinki Declaration; ethics approval was granted by the Institutional Review Board of Alexandra Hospital in Athens, Greece (Protocol code: 908/15.11.2019), and each participant gave informed consent.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: FZ has received honoraria for lectures and has served in an advisory role for AstraZeneca, Daiichi, Eli-Lilly, Merck, Novartis, Pfizer, and Roche. MAD has received honoraria from participation in advisory boards from Amgen, Bristol-Myers-Squibb, Celgene, Janssen, Takeda. The remaining authors (AK, AMK, MAP, KA, AN, GB, DT) declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

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Authors' contributions

FZ, MAD, and AK conceptualized the project and the methodology. AMK, MAP, GB, and DT were involved in data collection. AF was responsible for the immunohistochemical analysis and KA, with the support of MAP, performed the statistical analysis. AK and AMK interpreted the data and were the authors of the manuscript. FZ and MAD were actively involved in the interpretation of the results, providing important intellectual content. All authors provided critical feedback, contributed to the manuscript, and approved the final version in accordance with criteria established by the International Committee of Medical Journal Editors (ICMJE).

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Not applicable.

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Figures

Figure 1

TAZ and YAP1 immunohistochemical staining. A: No nuclear staining of the neoplastic cells (X200). B: Weak nuclear staining of some neoplastic cells (X200). C: YAP1/TAZ strong nuclear staining of the neoplastic cells (X50). D: YAP1/TAZ strong nuclear staining of the neoplastic cells (X200).

A.

B.

Figure 2

Overall survival (A) and disease-free survival (B) Kaplan-Meier curves.