Acta Biomed 2025; Vol. 96, N. 1: 16630 DOI: 10.23750/abm.v96i1.16630

Original article

Role of phosphatidylinositol 3 kinase in metastasis and grading of luminal breast cancer subtypes

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Abstract. *Background and aim:* The luminal subtype (HR+/HER2-) represents 70% of breast cancer cases. This subtype frequently involves alterations of the phosphatidylinositol 3-kinase (PI3K) pathway that result in tumor development, disease progression, and endocrine therapy resistance. This study aimed to examine the association between PI3K expression in luminal subtype breast cancer cells and metastasis in breast cancer patients in Makassar, Indonesia. *Methods:* A cross-sectional study was conducted to semi-quantitatively analyze PI3K expression in breast cancer patients. Staining intensities and percentages of carcinoma cell membranes stained relative to the total carcinoma cells were measured. Immunohistochemistry results were scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) according to the percentage of the area. Chi-square tests were performed to assess the relationships between PI3K and breast cancer grading and metastasis. *Results:* Breast cancer patients (74) who met the inclusion criteria for the study sample consisted of 30 (40.5%) patients with metastatic breast cancer and 44 (59.5%) with non-metastatic breast cancer. PI3K positivity was common in patients with high-grade malignant mammary carcinoma (75.9%; p-value 0.003 (≤0.05)) and those with metastasis (75.9%; p-value 0.015 (≤0.05)). Patients with PI3K-positive breast cancer had a 2.302 times greater probability of metastasis than those with negative PI3K values (95% CI 1.147–4.617). *Conclusions:* As the overexpression of PI3K increases, the histopathological grading of breast cancer and the tendency for distant metastasis escalate. (www.actabiomedica.it)

Key words: metastasis, breast cancer, phosphatidylinositol 3-kinase, HER2, luminal subtype, prognostic factors

Introduction

Breast cancer is the most common type of cancer worldwide, surpassing lung cancer as the leading cause of global cancer incidence in 2020 (1). Approximately 2.3 million new breast cancer cases, representing 11.7% of all cancer cases, were reported in that year, and 1 in 4 cancer cases and 1 in 6 cancer deaths were related to this form of cancer in most countries (159 out of 185 countries) with 685,000 deaths per year. Current breast cancer management requires multimodality therapy and personalized medicine (2). Research conducted at Wahidin Sudirohusodo Hospital in Makassar, Indonesia from January 2002 to 2019 identified that out of 7824 cancer patients divided into solid and non-solid cancer classifications, the solid cancer group had

an incidence of 79.3% and a death rate of 61.7% (1063 individuals) The majority of cancer cases occurred in females (3339 individuals, 42.7%), with the highest number in the 40-49 age group (2035 individuals, 26%), and the majority relating to breast cancer (1008 patients, 12.9%) (1). The causes of breast cancer are multifactorial; however, with the rapid development of diagnostic technology and innovation in cancer treatment, the morbidity and mortality rates can be reduced. Currently, the main method of determining breast cancer therapy types is through an immunohistochemistry (IHC) examination. Of the four molecular subtypes of breast cancer, the most common is luminal A (45%), followed by hormone receptor-positive (39%), luminal B (30%), and triple-negative breast cancer (9%) (3). Research conducted in the last decade has focused on specific targets associated with phosphatidylinositol 3 kinase (PI3K) expression toward cancer management. The importance of PI3K in the physiological processes of replication, growth, and survival of normal cells has been identified in various studies (4). In patients with breast cancer, excessive expression of PI3K causes uncontrolled cell growth resulting in cancer cells with high replication and metastasis abilities. Studies have shown PI3K overexpression in breast cancer patients (5,6). PI3K examination is required by clinicians to provide targeted therapy. The use of alpelisib, buparlisib, taselisib, and pictilisib increases progression-free survival (PFS) in advanced and metastatic breast cancer patients with increased PI3K expression (7). Luminal subtype breast cancer (HR+/HER2-) represents 70% of breast cancer cases. This subtype frequently involves the alteration of the PI3K pathway, which causes tumor development, disease progression, and endocrine therapy resistance (3). In Indonesia, and particularly in Makassar, PI3K has not been extensively studied in association with breast cancer. Therefore, the relationship between PI3K expression in luminal subtype cancer cells and metastasis in breast cancer patients in Makassar was examined.

Materials and Methods

This was a cross-sectional study conducted at Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar, Indonesia. Breast cancer patient tissue specimens were examined for PI3K expression at the Department of Pathological Anatomy, Faculty of Medicine, Hasanuddin University. The study was conducted from December 2022 to April 2023. Inclusion criteria for this study included female breast cancer patients aged 17 to 65 years with the luminal subtype, a normal BMI (18.5 – 22.9), metastatic or non-metastatic disease, and a willingness to participate in the study. Exclusion criteria included luminal subtype breast cancer patients with positive HER2/Neu IHC examinations, the presence of other malignancies (colorectal cancer, liver cancer, lung cancer, brain cancer), or tissue samples not suitable for IHC examination (discohesion, denaturation). PI3K expression was assessed semi-quantitatively by evaluating the staining intensity and percentage of stained carcinoma cell membranes and comparing the results to those of all carcinoma cells. Immunohistochemistry results were scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) according to the percentage of area (Figure 1). PI3K expression was considered overexpressed if staining expression (2+ to 3+) was found in ≥25% of tumor cells in cancer specimens. PI3K expression was considered not overexpressed if staining expression (0 to 1+) was found in <25% of cancer cells in tumor specimens.

Research procedure

All breast cancer patients included in the study provided informed consent, which included an explanation of the benefits and procedures of the research. Anamnesis was conducted to record patient information and examination results according to the research form prepared. Data in this study were divided based on histopathological grading (I, II, or III) and metastasis (presence or absence). Breast tissue samples were collected from patients under sterile conditions and placed in bottles containing 10% formalin buffer solution. Tissue specimens were prepared using paraffin blocks and observed under a microscope. The PI3 kinase p110 alpha antibody with GeneTex (Irvine, California, USA) catalog no. GTX100462 was examined immunohistochemically in all samples to assess the expression of PI3K membrane protein as positive or negative.

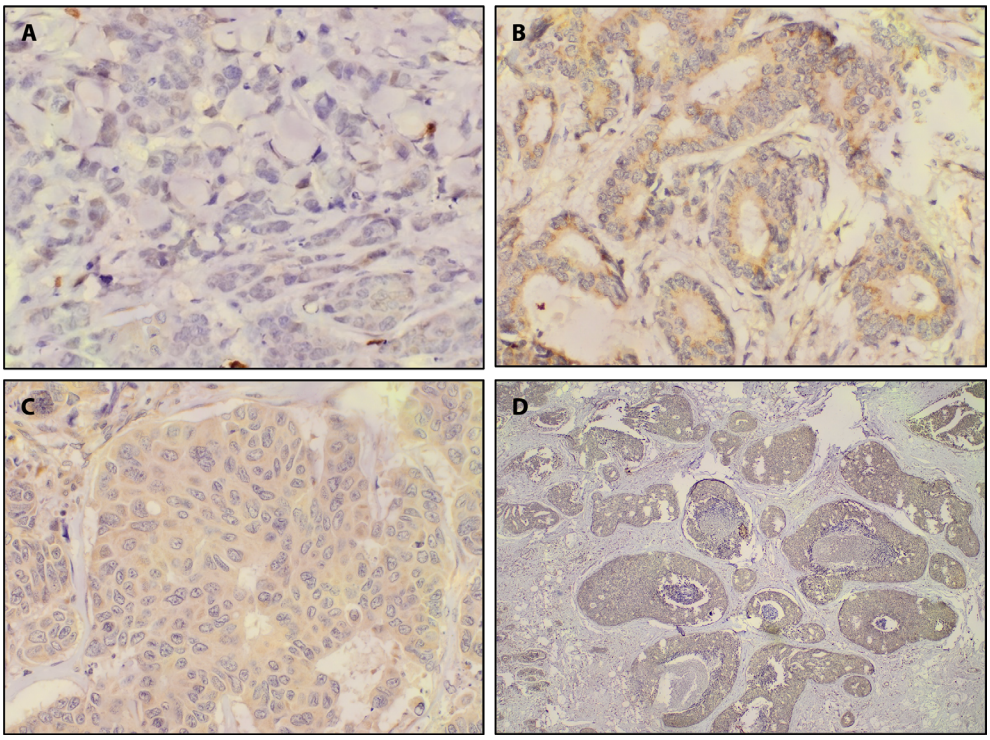

Figure 1. Shows representative immunohistochemistry staining of PI3K in breast cancer tissues. A) demonstrates negative PI3K immunostaining with no observable staining, B) demonstrates weak PI3K immunostaining, C) demonstrates moderate PI3K immunostaining, and D) exhibits strong PI3K immunostaining.

Statistical analysis

The data were analyzed using SPSS version 24.0 (Armonk, NY: IBM Corp.). A chi-square statistical analysis was conducted to assess the relationships between PI3K and the grading and metastasis of breast cancer. The values were considered statistically significant at p-values < 0.05.

Results

Of the 74 breast cancer patients included in this study, 30 (40.5%) had metastatic breast cancer and 44 (59.5%) had non-metastatic breast cancer. The characteristics of the patients are listed in Table 1.

The majority of patients were over 50 years old, (39, 52.7%), and 38 (51.3%) were pre-menopausal. The most common histopathological type, tumor grading, and subtype were invasive ductal mammary carcinoma, with 57 (77.02%) individuals, moderate grade, with 30 (40.5%) patients and luminal B subtype, with 49 (66.2%) participants, respectively. Positive PI3K expression was found in 42 (56.8%) patients, and 29 (39.2%) cases of breast cancer metastasis were observed, with the bones as the most common location (10 patients, 34.5%).

Table 2 shows that PI3K positivity was higher in breast carcinoma patients with a high-grade malignancy (75.9%). The chi square test indicated a p-value of 0.003 (p-value ≤ 0.05), signifying a significant association between PI3K and histopathological grading.

Table 3 shows that positive PI3K results were more prevalent in breast cancer patients with metastasis (75.9%) than in those without metastasis. The chi square test result indicates a p-value of 0.015 (p-value ≤ 0.05), signifying a significant association between PI3K and metastasis. Breast cancer patients with positive PI3K values have a 2.302 times greater chance of metastasis

Table 1. Characteristics of participants

Characteristic	n	%
Age (years)		
≤ 50	35	47.3
≥ 50	39	52.7
Menopausal Status		
Pre-menopause	38	51.3
Menopause	36	48.7
Histopathological Type		
Invasive Ductal Mammary Carcinoma	57	77.02
Invasive Lobular Mammary Carcinoma	12	16.22
Mucinous Mammary Carcinoma	5	6.76
Grading		
Low-Grade	15	20.3
Moderate-Grade	30	40.5
High-Grade	29	39.2
PI3K Expression		
Positive	42	56.8
Negative	32	43.2
Subtype		
Luminal A	25	33.8
Luminal B	49	66.2
Metastasis		
Yes	29	39.2
No	45	60.8
Metastasis Location		
Lung	8	27.6
Bone	10	34.5
Liver	3	10.4
Brain	1	3.4
Multiple	7	24.1

Table 2. PI3K expression profile with histopathological grading

PI3K Expression	Grading			p-value
	Low n (%)	Moderate n (%)	High n (%)	
Positive	10 (66.7)	10 (33.3)	22 (75.9)	0.003
Negative	5 (33.3)	20 (66.7)	7 (24.1)	

Note: *Chi Square*

compared to those with negative PI3K values (95% CI 1.147–4.617). The prevalence rate (PR) obtained in this study was 2.394; therefore, breast cancer patients with positive PI3K values had a 2.394 times greater risk of metastasis compared to those with negative PI3K values.

Discussion

In this study, the highest frequency of positive PI3K expression was found in Grade II cancers (moderate-grade malignancy; 30 cases, 40.5%). This finding contrasts with that of Siregar et al., who stated that most cases were classified as Grade III (50.8%), followed by Grade II (47.7%), and Grade I (1.5%) based on histopathological assessment (8). Those authors reported that their results aligned with previous research highlighting Grade III as the most common breast cancer grade in Indonesia, indicating the need for early detection programs and increased awareness (8,9). Positive PI3K expression was the most prevalent in this study, with 42 cases (56.8%). This corroborates the results of another study that stated that the PI3K pathway is a crucial signaling transduction pathway in cancer growth and development (10). This pathway plays roles in several functions, such as proliferation, cell growth, and tumor survival and PI3K activation has been identified in various cancer cases, including breast cancer. Excessive activation of this pathway leads to chemotherapy resistance and increased metastasis (10–14). The majority of low-grade malignancies had positive PI3K expression, with 10 respondents (66.7%), whereas the majority of moderate-grade malignancies had negative values (20 individuals, 66.7%). High-grade malignancies had mainly PI3K positive results with 22 respondents (75.9%). The test results indicate a p-value of 0.003, which is lower than the 0.05 significance level. This signifies a relationship between grading and PI3K, which agrees with the study by Lin et al., in which PI3K expression was associated with metastasis (p = 0.008) and high-grade malignancy (p = 0.01) (15). That study also indicated a direct relationship between PI3K gene expression and the size and grade of breast cancer tumors. Overall, PI3K plays a role in enhancing tumor development in breast cancer. Moreover, increased PI3K expression is associated with metastasis and poor cancer prognosis; thus, PI3K may be useful in the diagnosis, treatment, and prognosis of individuals with this disease.

Table 3. PI3K expression profile with metastasis

PI3K	Metastasis		95% CI	p-value	PR
	Yes n (%)	No n (%)			
Positive	22 (75.9)	20 (44.4)	2.302 (1.147-4.617)	0.015	2.394
Negative	7 (24.1)	25 (55.6)			

Note: *Chi Square*

However, further investigation is needed to support these claims (15,16).

The majority of patients with metastasis had positive PI3K expression (22 respondents, 75.9%) while those without metastasis mainly showed negative PI3K values (25 patients, 55.6%). The test results indicate a p-value of 0.015, which is less than the significance level of 0.05. Therefore, a relationship was evident between metastasis and PI3K. This aligns with the results of a study by Lin et al., which stated that PI3K gene expression in metastatic tumors was significantly increased when compared to the expression in patients without metastasis (15). High PI3K expression decreases overall survival when compared to low expression (p = 0.03) (3,15,17,18). A study on PI3K gene expression and its relationship with metastasis in patients with lung adenocarcinoma and breast cancer showed that increased gene expression was associated with cancer cell metastasis to lymph nodes (19,20). This finding is consistent with other research results indicating that PI3K plays a significant role in tumor tissue spread (10,21). The strength of this study lies in the large number of breast cancer patients and the ability to conduct IHC examinations in Makassar, which could promote this study as a pilot for other PI3K studies in that city. There are several limitations to this study that should be noted. Firstly, the measurement of dependent variables, particularly the assessment of distant metastasis, was conducted using chest X-rays, abdominal ultrasounds, and examinations based on patient complaints. Ideally, PET scans should have been used to ensure more accurate measurements. Additionally, the study utilized a cross-sectional design which prevented an assessment of whether PI3K overexpression in early-stage cancers would lead to distant metastasis, as patients presenting with early stages are rare. Furthermore, the number of cases analyzed in our study was relatively small and we acknowledge that the correlation analysis performed for the degree of PI3K expression was based on IHC rather than mutational analysis.

Conclusion

This study demonstrated a relationship between PI3K expression and histopathological grading in luminal subtype breast cancer. The results indicate that greater PI3K overexpression corresponds to higher histopathological grades of breast cancer. In addition, a relationship was found between PI3K expression and metastasis in luminal subtype breast cancer, whereby greater PI3K overexpression was associated with a higher tendency for distant metastasis. This was also evidenced by luminal subtype breast cancer patients with distant metastasis tending to have PI3K overexpression.

Acknowledgements: This research was supported by Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

Funding: This research received no external funding.

Ethic Committee: All research designs were reviewed and approved by the Health Research Ethics Committee of Dr Wahidin Sudirohusodo Hospital – Faculty of Medicine, Hasanuddin University (767/UN4.6.4.5.31/PP36/2022).

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership,

equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: YA (Concept, Design, Resources, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Search, Writing Manuscript). PRI (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), JP (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), IDR (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), SAS (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), NS (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), ES (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), BJN (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), AR (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), FRD (Analysis and Interpretation, Literature Search), and MF (Concept, Design, Analysis and Interpretation, Critical Review). All authors read and approved the final version of the manuscript.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Received: 20 November 2024
Accepted: 27 December 2024
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