

Research Article

Differentiation of Cutaneous Metastatic Breast Cancer from Cutaneous Adnexal Tumors Using Wnt9b Polyclonal Antibody Stain

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Abstract

Objective: The histologic diagnosis of cutaneous metastatic breast cancer can be challenging as the differentials can include primary cutaneous glandular neoplasms and metastases from other glandular neoplasms which present very similar on H&E. Many immunohistological markers including GATA3 and CK7 have been employed to screen for primary or metastatic breast cancer cells and because of this, we wanted to develop a stain capable of differentiating these diagnoses quickly and accurately.

Methods: We utilized 61 archived dermatopathology laboratory specimens of various benign and malignant cutaneous adnexal and breast tissues for analysis with a polyclonal Wnt9b antibody stain.

Results: The average staining in benign categories (cutaneous adnexal and benign breast tissue) as well as metastases from non-breast carcinomas was negative. Among the malignant cutaneous adnexal and metastatic breast tissues, a significant difference was observed in staining as adnexal carcinomas were weakly positive (0.53+) and primarily seen in the outer layer of glandular structures, while metastatic breast tissues were strongly positive (3.63+) ($P < 0.01$). The specificity in both adnexal and metastatic breast tissues was 100% while the sensitivity for adnexal carcinomas was 37% and metastatic breast was 94%. A larger sample size could greatly improve these values.

Conclusion: These results demonstrate that Wnt9b has specific staining for cutaneous metastatic breast cell nuclei and could be utilized as a diagnostic to differentiate from cutaneous adnexal tumors in routine dermatopathological applications.

Keywords: Metastatic Breast Carcinomas; Cutaneous Adnexal Tumor; Staining; Polyclonal Antibody; Histological Markers

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Introduction

The differentiation of cutaneous metastatic breast cancer and cutaneous adnexal tumors is critical as there may be histologic similarities, but treatment is drastically different [1-4]. Currently, the primary method to differentiate the two diagnoses is with careful histological review using hematoxylin and eosin staining along with immunohistological stains for tumor cell markers sometimes complemented by patient history [5]. This methodology can be prone to diagnostic errors. For this reason, the focus of our study is to evaluate a new immunohistological stain that could differentiate these two pathologies allowing for a more rapid and accurate diagnosis.

Many malignancies result from mutations in signaling throughout the WNT pathway which functions in cell replication regulation [6]. Some of the most common are loss-of-function mutations of Adenomatous Polyposis Coli (APC) or gain-of-function/over-expression mutations seen in β -catenin or Frizzled (Fzd) receptors [7]. These mutations in signaling can result in upregulation or switching of proteins used in the pathway and can be used as markers for tumorigenesis.

Commonly used specific markers within the WNT pathway include cytokeratin proteins, like CK1, which is part of the complex inhibiting β -catenin activity [8]. Related to it is CK7 which is one of the other cytokeratins that have been discovered and used in clinical practice as tumor markers. CK 7, 8 and 18-20 were identified in simple epithelia and CK7, specifically, has been utilized as a breast lineage marker in diagnostic distinctions similar to ours [9,10]. Similarly, GATA3, a transcription factor used in the immune system, is highly expressed in the hematopoietic compartment with essential roles in the development and function of T cells, B cells, CD1-restricted NK cells and innate-like lymphoid cells rendering it as a useful clinical diagnostic marker within tumors [10,11]. Both CK7 and GATA3 have been used routinely in clinical pathology to diagnose breast tumor origins, however, although both have been found to have high sensitivity, they tend to be lacking in specificity [10]. Another protein is Wnt which is the initiator of the WNT pathway activating the protein cascade by binding Fzd/LRP, ROR or RYK receptors on the cell membrane [8]. It is part of a family of proteins with 19 different gene products in humans that have roles in embryonic development, tissue homeostasis and have been implicated in tumor proliferation [10]. A study conducted by Lu, et al., found that Wnt9b was a more sensitive and specific marker for breast cancer compared to CK7 and GATA3 in samples of metastatic breast cancer with moderate to strong nuclear staining in the majority of both tumor and non-neoplastic breast epithelium [10].

In dermatopathological practice, especially, the diagnosis of cutaneous metastatic breast cancer versus cutaneous adnexal tumors presents a roadblock in differentiation due to the tissues presenting similarly on routine H&E stains. Even though they appear similar on H&E, the prognosis and treatment of each is vastly different and thus requires quick and accurate diagnosis by the pathologist. With this histological roadblock and these prior literature findings in mind, we wanted to explore the possibility that polyclonal antibody Wnt9b stain could be used in differentiating cutaneous metastatic breast tissue from cutaneous adnexal tumors or miscellaneous metastatic cancers with a higher level of sensitivity and specificity than prior routine stains (etc. GATA3).

Material and Methods

Patient Selection

The 61 specimens used in our study were derived from 56 patients (some were collected from two separate anatomic locations on a patient or had different diagnoses from the same patient) found in a computer search of laboratory archived dermatopathological specimens. Of the 61 total cases examined, 42 (69%) were female patients and 19 (31%) were male patients. There were four tissue samples of benign axillary tissue containing eccrine and apocrine glands of which two were male and two were female-derived. Of the 30 cutaneous adnexal tumor samples, eight (27%) were from males and 22 (73%) were from females. There were two cases of benign breast tissue (Fig. 1) which were both from female patients (100%) while the 16 metastatic breast cancer specimens (Fig. 1) were from 14 (88%) female patients and two (12%) male patients. Finally, the nine miscellaneous metastatic cancer tissue samples (Fig. 1) were derived from seven male patients (78%) and two female patients (22%). The age range overall and within each case grouping varied greatly with a range of 20 to 99 years old. Apocrine and eccrine glands in axillary skin were used as controls (Table 1).

Immunohistological Stain and Preparation

We employed an immunohistological stain made up of a rabbit polyclonal Wnt9b antibody (HPA058361, 1:100; MilliporeSigma, St. Louis, MO) utilized by Lu, et al., in their study on developing a specific marker for breast cancer. Tissue sections were cut at 5 μ m and oven-dried at 65°C for 45 min. Sections were then stained using a Leica Bond III stainer with epitope retrieval 2 (pH ~ 8) for 40 min. The sections were incubated with our antibody marker for 120 minutes followed by DAB staining with hematoxylin counterstain.

Grading was assigned by a percentage of nuclear staining observed. Negative (-) staining was denoted for tissues with 0% nuclear staining (Fig. 1), 1+ staining was denoted for 1-25% nuclear staining, 2+ staining for 25-50% nuclear staining, 3+ staining for 50-75% and 4+ staining for 75-100% (Fig. 1).

Statistical Methods

Staining intensity means were calculated and analyzed via a weighted one-way Analysis of Variance (ANOVA) for independent samples with a Tukey HSD test calculated via VassarStats (Vassar College, New York, NY).

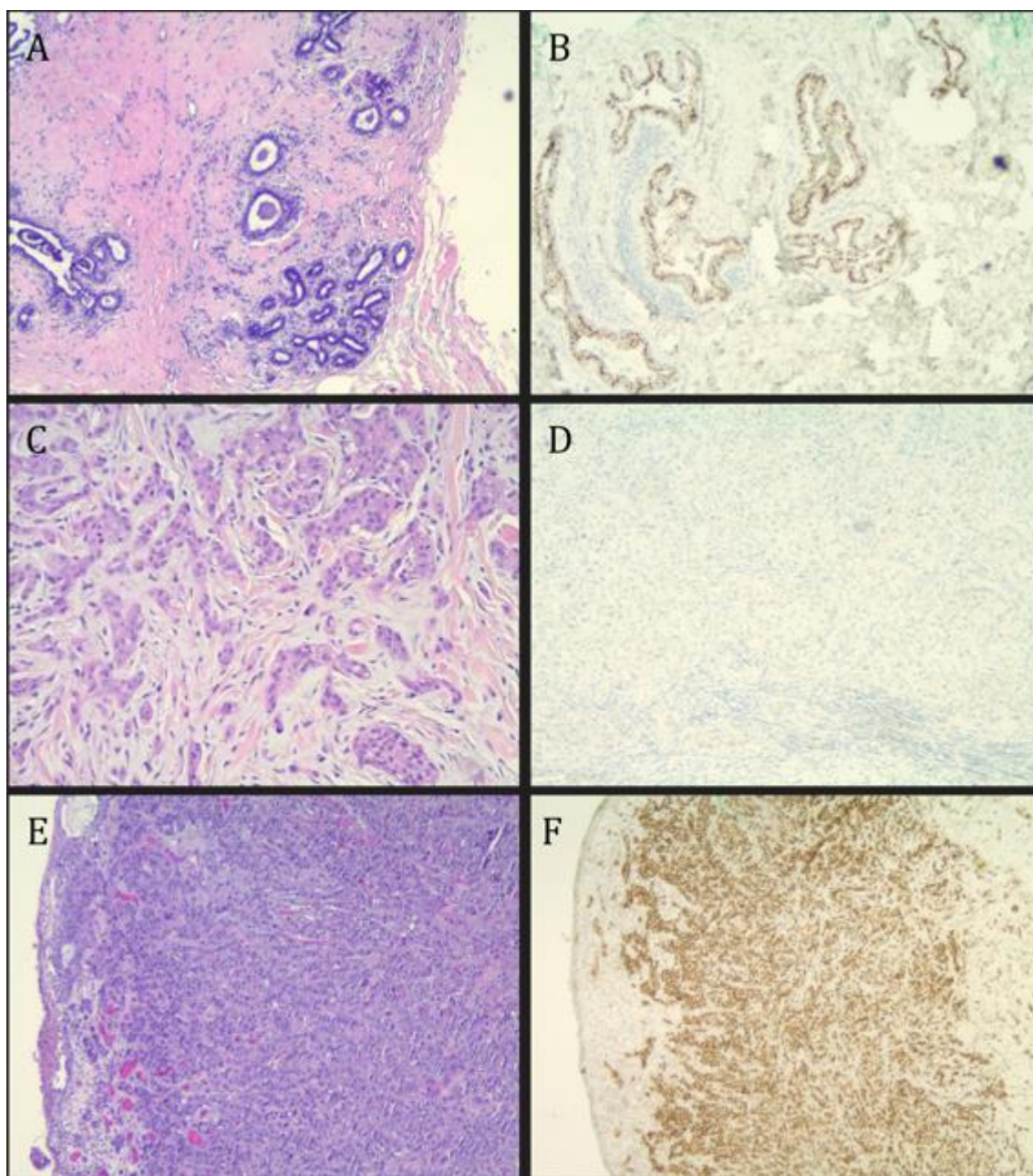


Figure 1: Staining of benign and malignant tissues using H&E or Wnt9b immunohistological stain. A: H&E stain of normal/benign breast tissue (40x); B: Wnt9b stain of normal/benign breast tissue with staining of inner luminal cell layer (40x). C: H&E stain of cutaneous urothelial metastatic tissue (100x); D: Negative Wnt9b stain of cutaneous urothelial metastatic tissue (100x); E: H&E stain of cutaneous metastatic breast tissue (40x); F: 4+ Wnt9b stain of cutaneous metastatic breast tissue (40x).

Classification			No. Cases, n	Sex, F / M	Stain Intensity (%)
Benign Adnexal Tissue	Benign Glands	Axilla	4	2 / 2	Apocrine: - (75%), 1+ (25%) Eccrine: 1+ (50%), 2+ (50%)
Cutaneous Adnexal Tumors	Hidradenoma		14	10 / 4	
		Scalp	2	1 / 1	- (50%), 1+ (50%)
		Face	4	4 / 0	- (25%), 1+ (50%), 3+ (25%)
		Neck	1	0 / 1	1+ (100%)
		Forearm	1	1 / 0	- (100%)
		Labia Majora	1	1 / 0	- (100%)
		Back	1	1 / 0	- (100%)
		Axilla	1	0 / 1	Apocrine: - (100%) Eccrine: 1+ (100%)
		Thigh	1	0 / 1	- (100%)
		Foot	2	2 / 0	- (50%), 1+ (50%)
	Syringocystadenoma papilliferum		7	3 / 4	
		Scalp	4	2 / 2	- (25%), 1+ (25%), 2+ (50%)
		Back	1	0 / 1	Glandular: 4+ (100%) Non-Glandular: - (100%)
		Neck	1	0 / 1	2+ (100%)
		Preauricular	1	1 / 0	- (100%)
	Sebaceous Hyperplasia	Cheek	1	1 / 0	- (100%)
	Eccrine Adenocarcinoma	Scalp, Nose	2	2 / 0	- (50%), 1+ (50%)
	Adnexal Carcinoma	Jawline	2	2 / 0	- (50%), 1+ (50%)
	Hidradenocarcinoma	Scalp	2	2 / 0	- (100%)
	Syringoma	Eye lid	1	1 / 0	- (100%)
	Microcystic adnexal carcinoma	Cheek	1	1 / 0	- (100%)
Benign Breast Tissue	Benign Cells	Breast	2	2 / 0	Inner Luminal Cells: - (50%), 2+ (50%) Outer Basal Cells: - (100%)
Cutaneous MetBC			16		
		Right Breast	2	2 / 0	4+ (100%)
		Left Breast	2	2 / 0	Tumor: 4+ (100%) Ducts: 1+ (50%)
		Scalp	4	3 / 1	- (25%), 3 (25%), 4+ (50%)
		Chest	4	3 / 1	3+ (50%), 4+ (50%)
		Sternum	1	1 / 0	4+ (100%)
		Cheek	1	1 / 0	4+ (100%)
		Neck	1	1 / 0	4+ (100%)
		Flank	1	1 / 0	4+ (100%)
Misc Metastatic Carcinomas	Prostate	Thigh	2	0 / 2	- (100%)
		Inguinal	1	0 / 1	- (100%)
	Urothelial	Inguinal Crease	1	0 / 1	- (100%)
	Renal	Postauricular	1	0 / 1	- (100%)
	Colon	Scalp	1	0 / 1	- (100%)
		Back	1	0 / 1	- (100%)
	Endometrial	Thigh	1	1 / 0	- (100%)
		Labia Majora	1	1 / 0	- (100%)

Note: MetBC = Metastatic Breast Cancer; Stain intensity refers to the percentage of nuclei staining within a case sample with negative or "-" (0%), 1+ (0-25%), 2+ (25-50%), 3+ (50-75%) and 4+ (75-100%)

Table 1: Wnt9b antibody staining of cutaneous tissue samples.

Results

For the cutaneous adnexal tumor cases, we included four cases of benign axillary apocrine and eccrine gland tissue for comparison. Of these cases, all four featured apocrine tissue but only one (25%) stained very weakly positive within its nuclei while the rest were negative. Two (50%) of the axillary tissue cases featured eccrine glands that showed focal weak staining of (2+) and (1+). There were 30 adnexal neoplasms of which 14 were hidradenomas of various cutaneous locations, seven were syringocystadenoma papilliferums of the scalp (4), back (1), neck (1) or preauricular (1), two hidradenocarcinomas of the scalp, an eccrine adenocarcinoma of the scalp and from the nose and two adnexal carcinomas of the jawline. Of all the malignant adnexal cases, only one case had any strongly positive staining and it was a syringocystadenoma papilliferum of the back in which the glandular area stained strongly (4+) while the non-glandular area was negative. One case of hidradenoma of the cheek

showed some moderately positive staining (3+). There were three cases of syringocystadenoma papilliferum (two of the scalp and one of the neck) that had focal positive staining (2+) but with notably peripheral cell-staining distribution. The rest of the malignant adnexal cases were either weakly staining (1+) or negative (Table 1).

For the cutaneous metastatic breast cancer cases, we included two cases of benign breast tissue for comparison. One case was completely negative while the other had negative outer basal cells but slightly positive (2+) inner luminal cells. A marked contrast was seen from these benign tissue samples compared to the cutaneous metastatic breast cancer tissues. Of the 16 metastatic breast cases, two (12.5%) were from the cutaneous tissue of the right breast and two (12.5%) from the left breast, four (25%) were from the scalp, four (25%) from the chest, one (6%) from the sternum, one (6%) from the cheek, one from the neck (6%) and one from the right flank (6%). 13 (81%) of the metastatic breast cancer cases stained strongly positive (4+), two (12.5%) moderately positive (3+) and one (6%) negative. Of note, one left cutaneous breast case showed strongly positive (4+) staining within the tumor tissue but weakly positive (1+) staining in the ductal tissue (Table 1).

We included nine miscellaneous metastatic cancer tissue cases to confirm sensitivity and specificity for metastatic breast tissue rather than general metastatic cancer tissues. Tissues were derived from metastatic prostate, urothelial, renal, colon and endometrial carcinomas. All nine (100%) samples stained negatively (Table 1).

VassarStats also gave the average staining intensity per tissue category so we could compare the overall staining intensity between the different tissue types. The benign adnexal, benign breast and miscellaneous metastatic samples were overall negative in staining. The cutaneous adnexal tumor tissue samples had an average of (0.53+) staining while the cutaneous metastatic breast tissue samples had an average stain of (3.63+), staining the most robustly out of the categories (Table 2). A weighted one-way ANOVA with a Tukey test was conducted on these means and found an overall significant P-value of <0.0001 for all samples together and a significant difference ($P < 0.01$) between the malignant cutaneous adnexal and breast staining samples. Sensitivity and specificity were calculated by comparing positive or negative staining between the benign and malignant samples of each category (adnexal or metastatic breast). The 30 cutaneous adnexal tumor samples showed that the Wnt9b stain had 37% sensitivity (11/30) and 100% specificity (4/4) while the 16 breast samples had 94% sensitivity (15/16) and 100% specificity (2/2). The miscellaneous metastatic cancer tissues had 0% sensitivity.

Tissue Type	Overall Average Staining Intensity (n)
Benign Adnexal Tissues	Negative (4)
Adnexal Tumor Tissues	0.53+ (30)
Benign Breast Tissues	Negative (2)
Metastatic Breast Tissues	3.63+ (16)
Miscellaneous Metastatic Cancer Tissues	Negative (9)

Note: Averages taken from apocrine tissue in both adnexal categories, outer basal cells in benign breast, and tumor cells of metastatic breast tissue as these areas were most consistently observed

Table 2: Wnt9b antibody staining of benign vs malignant cutaneous tissue samples.

Discussion

There exists a plethora of diagnostic immunohistological markers used in clinical practice today to identify aspects of both benign and malignant lesions found throughout the body. These markers can range from identifying the origin tissue of a tumor to identifying pathways and receptors the tumor cells are using to enhance tumorigenesis. We utilized Wnt9b staining to differentiate cutaneous adnexal tumors from cutaneous metastatic breast tumors.

Several markers are used in routine clinical diagnostics that are sensitive and specific for breast tissues. GATA3 is utilized as a diagnostic marker for metastatic breast cancers with strong sensitivity but weaker specificity compared to other markers in breast tissues, like Wnt9b, due to its frequent expression in urothelial cancer and other tumors [10]. Cytokeratins have also played a large role as tumor markers in various anatomical tissues. Some examples include CK 7, 8 and 18-20 being identified in simple epithelia; CK5, 14 and 17 in basal cells; CK7, 8, 18 and 19 being observed in breast carcinomas with expression of CK5/6, 14 and 20 correlating with a higher tumor grading [9,10,12,13]. CK7 is utilized as a primary and metastatic breast cancer marker because

of its high sensitivity but it too, has been found to have weaker specificity compared to other markers in breast tissues like GATA3 [10]. The WNT pathway is critical to cell proliferation and tissue development throughout the body, including in the adnexal and breast tissues, so finding a WNT marker specific to just one of the two tissues would be key to their differentiation [15,16].

The study conducted by Lu, et al., describes a marker within the WNT pathway more specific to breast cancer tissue than any of the previous diagnostic markers used in clinical and/or research settings: Wnt9b. They found the marker to have similar sensitivity to GATA3 and CK7 but with increased specificity rendering it more useful for primary and metastatic breast cancers [10]. Lu, et al., study was novel with the introduction of the Wnt9b marker but shared similarities with many other previously published studies in that they were comparing sensitivity and specificity of cytokeratin or WNT markers on various tumor and benign tissues. However, no previous literature had investigated the use of Wnt9b as a diagnostic marker between two different types of malignant tissues; only between subtypes of malignant tissues (Lu, et al., comparing primary and metastatic breast tissue). Our study is also novel in the fact that we are investigating cutaneous tumor tissues while others were focusing on major internal organ primary tumors and metastases. With all of this in mind, we wanted to pursue the application of Wnt9b as a diagnostic marker to better discern cutaneous adnexal tumors from cutaneous metastatic breast cancers, both previously very difficult to distinguish histologically, with unmatched sensitivity and specificity.

Through our investigation, we found that the Wnt9b polyclonal antibody had significant ($P < 0.01$) staining with 37% sensitivity and 100% specificity in the cutaneous adnexal tumor samples, 94% sensitivity and 100% specificity in the cutaneous metastatic breast tissue samples and 0% sensitivity in the miscellaneous metastatic cancer tissues. With the high sensitivity and specificity values, our results support prior studies' data showing Wnt9b's expression in malignant breast tissues. In comparison to previous studies, Lu, et al., found both GATA3 and Wnt9b to have a sensitivity of 98.7% but a specificity of 87.3% for GATA3 and 96.8% for Wnt9b toward primary and metastatic breast tissues. The sensitivity in their study was higher, however, our specificities were stronger. The same goes for other similar studies: One conducted by Rollins-Raval, et al., found that p63, mammaglobin and the basal cell cytokeratins (CK5, 14 and 17) were all individually able to differentiate cutaneous metastatic breast lesions from sweat gland carcinomas but when combined were 100% sensitive and 91% specific in differentiating the two neoplasms [13]. Once again, compared to our use of Wnt9b, their sensitivity is stronger but their specificity was weaker. A study by Mentrikoski and Wick looked into a similar but opposite distinction testing immunohistochemical stains to differentiate cutaneous metastatic breast carcinoma from primary sweat gland carcinoma and found statistically significant differences in specificity for p63, CK5/6 and D2-40 markers [14]. They reported sensitivities of 81%, 71% and 52% respectively and specificities of 94% across the board for primary sweat gland carcinoma tissues [14]. Although their differentiation focus was toward adnexal carcinomas rather than cutaneous metastatic breast carcinomas, the aims of their study still closely resemble ours and the markers used come from similar biological origins. Despite this, our markers still showed markedly higher sensitivity and specificity in comparison. Wnt9b may be a specific and promising biologic marker and could prove to be the adjunct needed to achieve higher diagnostic accuracy in this clinical scenario.

Our study is not without limitations. Even though we achieved statistical significance between cutaneous adnexal and metastatic breast cancer staining, it is worth noting that the power of our study is lacking due to a smaller sample size (61) and that a larger sample size could greatly improve the study's power as well as the sensitivity and specificity percentages. Similarly, the specificity values obtained may be exacerbated by the fact that our control groups were also rather small (4 and 2). Nevertheless, the findings of our study show great promise in the use of Wnt9b as a dermatopathologic diagnostic marker and subsequent studies replicating our methods with larger sample sizes could further validate its future use in clinical settings.

Conclusion

The results of our study build continued evidence for the use of Wnt9b being a malignant breast tissue-specific stain as previously explored by Lu, et al., Furthering this notion, with significantly weaker staining in cutaneous adnexal tumor tissues, it seems that this stain could also be used in diagnostic pathological applications for differentiating cutaneous malignancies of the breast with high sensitivity and specificity as we and others have explored. This differential staining observed by the Wnt9b polyclonal antibody not only provides a novel diagnostic test for the dermatopathology field but also suggests further future capabilities of WNT pathway proteins for their use in targeted tissue diagnostic applications.

Conflicts of Interests

The authors declare that there is no conflict of interest for this paper.

Ethical Statement

Not applicable.

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References

1. Tan AR. Cutaneous manifestations of breast cancer. *Seminars in Oncology*. 2016;43(2016):331-4.
2. Płachta I, Kleibert M, Czarnecka AM, Spalek M, Szumera-Ciećkiewicz A, Rutkowski P. Current diagnosis and treatment options for cutaneous adnexal neoplasms with apocrine and eccrine differentiation. *Int J Mol Sci*. 2021;22(10):1-44.
3. Sariya D, Ruth K, Adams-McDonnell R. Clinicopathologic correlation of cutaneous metastases: Experience from a cancer center. *Arch Dermatol*. 2007;143(5):613-20.
4. Danialan R, Mutyambizi K, Aung PP, Prieto VG, Ivan D. Challenges in the diagnosis of cutaneous adnexal tumours. *J Clin Pathol*. 2015;68:992-1002.
5. Müller CS, Körner R, Takacs FZ, Solomayer EF, Vogt T, Pfoehler C. Metastatic breast carcinoma mimicking a sebaceous gland neoplasm: A case report. *J Med Case Reports*. 2011;5(428):1-5.
6. Kang Z, Xu F, Zhang QA, Wu Z, Zhang X, Xu J, et al. Oncogenic mutations in extramammary paget's disease and their clinical relevance. *Int J Cancer*. 2013;132(4):824-31.
7. Nie X, Liu H, Liu L, Wang Y, Chen W. Emerging roles of wnt ligands in human colorectal cancer. *Front Oncol*. 2020;10(1341):1341.
8. Holstein TW. The evolution of the wnt pathway. *Cold Spring Harbor Perspectives in Biology*. 2012;4(7):1-17.
9. Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. *Clinical Biochemistry*. 2004;37(7):529-40.
10. Lu S, Yakirevich E, Yang D, Xiao Y, Wang LJ, Wang Y. Wnt family member 9b (Wnt9b) is a new sensitive and specific marker for breast cancer. *The American J Surgical Pathol*. 2021;45(12):1633-40.
11. Wan YY. GATA3: A master of many trades in immune regulation. *Trends in Immunol*. 2014;35(6):233-42.
12. Shao M, Chan SK, Yu AMC. Keratin expression in breast cancers. *Virchows Arch*. 2012;461(3):313-22.
13. Rollins-Raval M, Chivukula M, Tseng GC, Jukic D, Dabbs DJ. An immunohistochemical panel to differentiate metastatic breast carcinoma to skin from primary sweat gland carcinomas with a review of the literature. *Arch Pathology & Laboratory Medicine*. 2011;135(8):975-83.
14. Mentrikoski MJ, Wick MR. Immunohistochemical distinction of primary sweat gland carcinoma and metastatic breast carcinoma. *Am J Clin Pathol*. 2015;143(3):430-6.

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