Genetic Changes of Wnt Pathway Genes Are Common Events in Metaplastic Carcinomas of the Breast

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Abstract **Purpose:** Metaplastic carcinomas are distinct invasive breast carcinomas with aberrant nonglandular differentiation, which may be spindle, squamous, or chondroid. The limited effective treatments result from the lack of knowledge of its molecular etiology. Given the role of the Wnt pathway in cell fate and in the development of breast cancer, we hypothesized that defects in this pathway may contribute to the development of metaplastic carcinomas.

Design: In 36 primary metaplastic carcinomas, we comprehensively determined the prevalence of and mechanism underlying β-catenin and Wnt pathway deregulation using immunohistochemistry for β-catenin expression and localization and mutational analysis for *CTNNB1* (encoding β-catenin), *APC, WISP3, AXIN1*, and *AXIN2* genes. By immunohistochemistry, normal β-catenin was seen as membrane staining, and it was aberrant when >5% of tumor cells had nuclear or cytoplasmic accumulation or reduced membrane staining.

Results: By immunohistochemistry, aberrant β-catenin was present in 33 of 36 (92%) cases, revealing deregulation of the Wnt pathway. *CTNNB1* missense mutations were detected in 7 of 27 (25.9%) tumors available for mutation analyses. All mutations affected the NH_2-terminal domain of β-catenin, presumably rendering the mutant protein resistant to degradation. Two of 27 (7.4%) tumors had mutations of *APC*, and 5 (18.5%) carried a frame shift mutation of *WISP3.* No *AXIN1* or *AXIN2* mutations were found.

Conclusions: Activation of the Wnt signaling pathway is common in this specific subtype of breast carcinoma. The discovery of *CTNNB1, APC*, and *WISP3* mutations may result in new treatments for patients with metaplastic carcinomas of the breast.

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Metaplastic mammary carcinomas are a histologically heterogeneous and unique group of tumors defined by the presence of a glandular and a nonglandular component (1–3). The nonglandular component results most of the times from mesenchymal differentiation, including cells with spindle, osseous, or cartilaginous features. Metaplastic carcinomas are almost invariably negative for hormone receptors and do not exhibit HER-2/neu overexpression. Despite advances in the understanding of the molecular mechanisms that underlie the development of invasive carcinomas of the breast, the molecular events leading to metaplastic carcinomas remain unknown. As a consequence, there are currently no effective chemotherapeutic treatment options for patients with metaplastic carcinomas, and, with the exception of the rare low-grade pure spindle cell carcinoma, these tumors have a guarded prognosis.

The *Wnt* signaling pathway has long been implicated in mammary gland development and carcinogenesis. Activation of the *Wnt* pathway in breast cells results in cell fate determination and in an epithelial to mesenchymal transition leading to invasion and metastasis. β-Catenin is a critical regulatory gene of the *Wnt* signaling pathway. When the *Wnt* signaling pathway is activated, β-catenin translocates from the membrane and accumulates in the nucleus where it interacts with members of the lymphoid enhancer factor/T-cell factor family of transcriptional activators as a critical intermediate in signal transduction pathways. Serine and threonine phosphorylation regulate its stability, targeting it to degradation through an adenomatous polyposis coli (APC)–mediated proteosomal pathway (4). A body of data support a role for *β-catenin* as an oncogene whose deregulation or mutational activation can lead to cancer (5).

Unlike colorectal carcinoma, hepatocellular carcinoma, and other tumors that have a high frequency of mutations of *β-catenin*, *APC*, and other critical Wnt pathway genes, mutations are very rare to nonexistent in breast cancer (6).

In an effort to begin to understand the molecular pathogenesis of this special type of breast cancer, we hypothesized that defects in the Wnt signaling pathway genes and proteins are responsible for the altered differentiation program characteristic of metaplastic carcinomas of the breast. For this, we collected 36 primary metaplastic carcinomas and carried out

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a comprehensive molecular analysis of several genes encoding proteins known to function in the Wnt signaling pathway. We found that β-catenin protein is aberrantly expressed in nearly all metaplastic carcinomas. β-Catenin deregulation was attributable in a group of cases to mutation of the *CTNNB1* gene itself and less frequently to inactivating mutations in the *APC* gene, or the Wnt-1 induced secreted protein 3 gene (*WISP3*). Our findings provide the first evidence for Wnt pathway defects in metaplastic carcinomas of the breast, and pave the way to explore urgently needed therapeutic interventions for patients with this unique and aggressive form of breast cancer.

Materials and Methods

Tumor samples. A total of 36 metaplastic carcinomas were analyzed. All slides were obtained with Institutional Review Board approval from the Surgical Pathology files at the University of Michigan. H&E-stained slides of formalin-fixed, paraffin-embedded tumors were analyzed by light microscopy independently and blindly by two pathologists (M.J.H. and C.G.K.) and a median of five slides were reviewed for each case. All metaplastic carcinomas were classified according to published accepted criteria used in clinical practice (2, 3). They were classified by the architectural pattern and the presence of epithelial and or heterologous elements. The metaplastic carcinomas were graded on the basis of the sarcomatoid component as grade 1 (low), grade 2 (intermediate), or grade 3 (high) following described criteria (7). In all cases, the diagnosis of metaplastic carcinoma was confirmed by positive cytokeratin staining including a cytokeratin cocktail (AE1/AE3, CAM5.2) and/or a high molecular weight cytokeratin stain (34βE12). Clinical and pathologic features including tumor stage (I-IV), tumor size, lymph node metastasis, and distant metastasis were available for the cases.

DNA preparation. Twenty-seven metaplastic carcinomas had blocks available for mutation analyses. Primary tumor tissues were manually microdissected before nucleic acid extraction to ensure that each tumor sample contained at least 70% tumor cells by an experienced pathologist in the study (D.T.). Several areas of the tumors were microdissected. H&E-stained sections of all tumor tissues were used as dissection guides. Each area of interest (e.g., glandular, spindle, cartilaginous, squamous, and osseous) was identified by two pathologists (C.G.K. and M.J.H.) and one to two 1-mm-diameter punches of these areas were obtained from the corresponding formalin-fixed, paraffin-embedded block. Genomic DNA was isolated using the commercially available Nucleon DNA extraction and purification kit following the manufacturer's instructions (Amersham Lifesciences).

PCR and sequencing. The PCR primers, amplicon size, and annealing temperatures used for each reaction are specified in Table 1. *CTNNB1* exon 3 was amplified from genomic tumor DNA using a forward primer located at the 5′ portion of the exon and a reverse primer at the 3′ end of the exon. Several tumor samples with known *CTNNB1* mutations (a kind gift of Drs. K.R. Cho and F.R. Fearon, University of Michigan, Ann Arbor, MI) were amplified and sequenced in parallel as positive controls. PCR for *APC* exon 15 was done with genomic DNA as previously described (8). *AXIN1* and *AXIN2* mutations were investigated as described by Webster et al. (9) and Wu et al. (10). PCR for *WISP3* was done on exon 4 encoding a domain associated with cell attachment as described by Thorstensen et al. (11). All PCR reactions were carried out in a final volume of 50 μL using Platinum PCR supermix (Invitrogen) and 200 nmol/L primers. After an initial denaturation and Taq DNA polymerase activation at 95°C for 10 min, templates were amplified for 35 cycles (94°C for 1 min, annealing temperature for 1 min, followed by chain extension at 72°C for 2 min), followed by a 10-min extension at 72°C. PCR products were visualized on 2% agarose gels and purified with Wizard SV PCR clean-up kit (Promega). Amplicons were sequenced directly in both directions at the University of Michigan Medical Center DNA sequencing core using an ABI 377 DNA sequencer (ABI). Chromatograms were downloaded directly to CodonCode Aligner software (version 1.6.3) and the sequence compared with reference sequence downloaded from National Center for Biotechnology

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Table 1. Primer sequences and annealing temperatures for PCR

Gene	Ref. seq	Exon	Function	Forward primer	Reverse primer	Annealing temperature (°C)
WISP3	NM_003880	4	Cell attachment domain	GATAGAGTGATAAATTAGACCATCGGCT	CATTGGTCACCCTGTTAGATATTCC	55
CTNNB1	NM_001904	3	GSK3β regulatory domain	ATGGAACCAGACAGAAAAGCGGC	GCTACTTGTTCTTGAGTGAAG	58
APC	NM_000038	15a	β-Catenin regulatory domain	CAGACTTATTGTGTAGAAGA	CTCCTGAAGAAAATTCAACA	52
APC		15b	β-Catenin regulatory domain	AGGGTTCTAGTTTATCTTCA	TCTGCTTGGTGGCATGGTTT	52
APC		15c	β-Catenin regulatory domain	GGCATTATAAGCCCCAGTGA	TGGCTCATCGAGGCTCAGAG	52
APC		15d	β-Catenin regulatory domain	ACTCCAGATGGATTTTCTTG	GGCTGGCTTTTTTGCTTTAC	52
AXIN1	NM_003502	2a	APC binding	CGTCATCGTGAGTCTTGTCT	TCAGCCCACTTCAAGTATGG	55
AXIN1		2b	APC binding	AAGGTGAGACTTCGACGG	ATAGTGGCCTGGATTTCGGT	55
AXIN1		2c	APC binding	AACAATGGCATCGTGTCCCG	TGTCTCCAGGAGCAGCTTCT	55
AXIN1		2d	APC binding	CCTGCCGACCTTAAATGAAG	GGACATCCGGTGTGGGTTAA	55
AXIN1		3	GSK3β binding	ACAGGTGGAGATGTTGGTC	ACACATTGCTGTCCTCAAGG	55
AXIN1		4	GSK3β binding	ATCACCTGTGTGCACGTGT	ACTGGCCACTTGCAGATG	55
AXIN1		5	GSK3β binding	AGCACACGCTTTCCCTTCA	CCCATGAAGAAGCATCAGGAC	55
AXIN1		6a	β-Catenin binding	TGGGTCAGGCCTGATGCTTTT	ACTGGCATCTTGGCCACGT	55
AXIN1		6b	β-Catenin binding	AGCATCCTGGACGAGCACGT	TGCAGAAGGCCAGAACCT	55
AXIN2	NM_004655	7	DSH binding	CAAAGCACAAAAAAGGCCTAC	GATTCCTGTCCCTCTGCTGAC	60

Abbreviation: GSK3β, glycogen synthase kinase 3β.

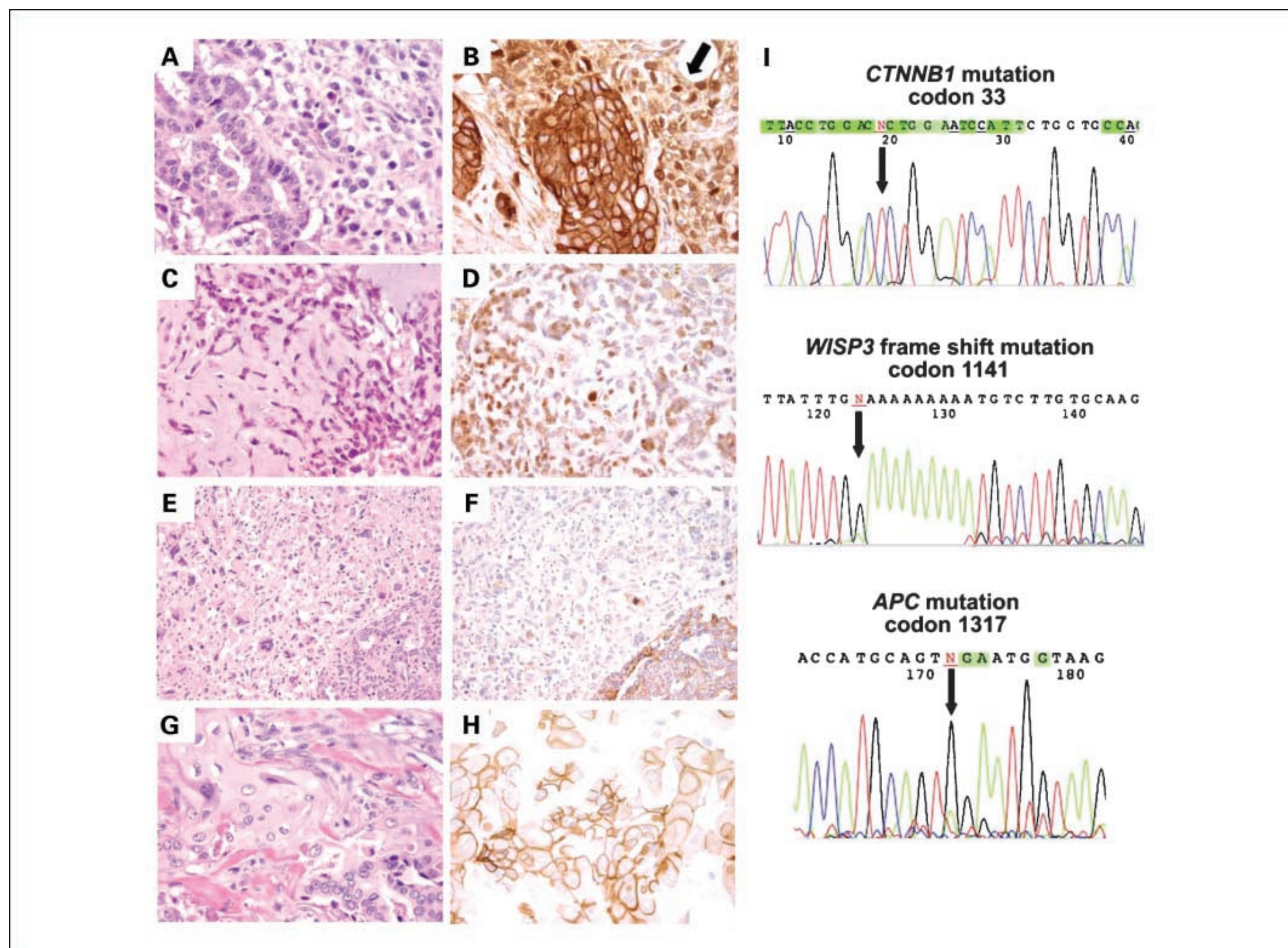

Fig. 1. Immunohistochemical staining of β-catenin in metaplastic carcinomas of the breast, and representative mutations on *CTNNB1, APC*, and *WISP3* genes. *A,* metaplastic carcinoma with glandular and spindle cell differentiation; *B,* same tumor showing nuclear accumulation of β-catenin in a region of the tumor with spindle cell differentiation (*arrow*). Note the membrane-associated β-catenin immunoreactivity in the epithelial component. *C,* metaplastic carcinoma with chondroid differentiation; *D,* this tumor shows prominent nuclear and cytoplasmic immunoreactivity for β-catenin in the vast majority of neoplastic cells. *E,* metaplastic carcinoma with glandular elements and highly atypical spindle cells; *F,* note the membranous expression β-catenin protein in the glandular elements (*bottom right*) in stark contrast to the nearly absent β-catenin expression in the malignant spindle cells. *G,* metaplastic carcinoma with squamous differentiation; *H,* this tumor shows membrane-associated localization of β-catenin immunoreactivity without detectable nuclear immunoreactivity. *I*, representative examples of *CTNNB1, WISP3*, and *APC* gene mutations found in these tumors. For the APC mutation, the G to A results in a stop codon. Magnification, ×400.

Information. All presumptive mutations were reamplified and resequenced from the original tumor DNA.

Immunohistochemical analysis of β-catenin. Immunohistochemical analyses were carried out at the University of Michigan Histology and Immunohistochemistry Core. Five-micrometer sections of formalin-fixed, paraffin-embedded tissues were mounted on plus slides, deparaffinized in xylene, and then rehydrated with distilled H_2O through graded alcohols. Antigen retrieval was enhanced by microwaving the slides in citrate buffer (pH 6.0; Biogenex) for 10 min. Endogenous peroxidase activity was quenched by incubation with 6% hydrogen peroxide in methanol, and then the sections were postfixed in 10% buffered formalin, washed, and blocked with 1.5% normal horse serum for 1 h. Sections were then incubated with a mouse monoclonal anti–β-catenin antibody (BD Biosciences) at a dilution of 1:400 for 30 min at 4°C. Slides were washed in PBS and then incubated with a biotinylated horse anti-mouse secondary antibody for 30 min at room temperature. Antigen-antibody complexes were detected with the avidin-biotin peroxidase method using 3,3′-diaminobenzidine as a chromogenic substrate (Vectastain ABC kit, Vector Laboratories). Immunostained sections were lightly counterstained with hematoxylin and then examined by light microscopy. Immunostaining was assessed, following previously published methods (10), as "normal" when β-catenin was seen as crisp membrane staining, or "aberrant" when >5% of tumor cells had nuclear or cytoplasmic accumulation, or reduced or absent membrane staining.

Results

Histopathologic and clinical features. All patients were female, with a median age of 60 years (range, 33-89 years). Of the 36 metaplastic carcinomas, 28 tumors had spindle and/or squamous areas, 6 had chondroid differentiation, and 2 had osseous differentiation (Fig. 1). Table 2 summarizes the clinical and histologic features of the metaplastic carcinomas. Of the cases with available staging information, 4 tumors were stage I, 12 stage II (9 IIA and 3 IIB), 1 stage III, and 5 stage IV. When present, distant metastases were seen in the lung parenchyma, pleura, brain, and vertebrae. Of the 36 metaplastic

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carcinomas, none had histologic grade 1, 11 (30.6%) had grade 2, 22 (61.1%) were grade 3, and in three we were unable to assess the histologic grade. The grade 2 spindle cell metaplastic carcinomas were characterized by elongated cells with minimal to moderate cytologic atypia and rare or no mitoses. In contrast, the grade 3 spindle cell carcinomas exhibited pleomorphism, hyperchromasia, increased cellularity, and numerous atypical mitoses. The neoplastic cells in both grade 2 and grade 3 spindle cell carcinomas infiltrated adjacent mammary and adipose tissues and were interrupted by dense collagen bands. One of the metaplastic carcinomas with squamous cell differentiation also showed spindle cell metaplasia; the other consisted entirely of squamous elements. Those tumors with chondroid and osseous differentiation exhibited the range of cytologic and architectural features seen in chondrosarcomas and osteosarcomas. These included cartilage and bone formation and a range of cytologic atypia, cellularity, and mitotic activity.

Aberrant localization of β-catenin in primary metaplastic carcinomas of the breast. Aberrant β-catenin protein expression was found in 33 of 36 (91.7%) metaplastic carcinomas (Tables 2 and 3; Fig. 1). The remaining 3 (8.3%) tumors had normal β-catenin protein expression characterized by crisp membrane staining. Primary tumors with aberrant nuclear and cytoplasmic accumulation of β-catenin frequently showed reduced or absent membrane staining. Similar to previously noted results in tissues and cell lines (10), aberrant β-catenin accumulation was typically noted in many, but not all, neoplastic cells within a given tumor.

CTNNB1, APC, AXIN1, AXIN2, ***and*** **WISP3** ***mutational analyses.*** Table 3 shows in detail the results of the sequence analysis of Wnt pathway genes in the 27 cases of metaplastic carcinoma with available tissue. Somatic missense mutations in *CTNNB1* sequences encoding the NH_2-terminal portion of β-catenin were identified in 7 of 27 (25.9%) tumors available for mutation analyses. *CTNNB1* mutations affected serine or immediately adjacent residues in the presumptive glycogen synthase kinase 3β regulatory motif at the β-catenin NH_2 terminus. Three mutations were found at codon 33 (TCT-TGT: Ser-Cys, two cases; TCT-ACT: Ser-Thr, one case). We found one mutation at codon 37 (TCT-TAT: Ser-Tyr), and in one case at codon 42 (ACA-AGA: Thr-Arg; Table 3; Fig. 1). *CTNNB1* mutations were more frequent in the mesenchymal cells when compared with the epithelial cells. Of the seven metaplastic carcinomas with *CTNNB1* mutations, three had mutations only in the spindle cell component, one in the osteosarcomatous component, and one in a glandular component.

Two metaplastic carcinomas had mutations within exon 15 at codon 1317 of the *APC* gene. This area, located at the COOH-terminal portion of the *APC* gene, is responsible for *CTNNB1* regulation (8, 12). Both mutations found resulted in the conversion of an arginine residue to a stop codon (A to T: Lys to stop; Fig. 1), which leads to a truncated protein without *CTNNB1* regulatory activity. These two *APC* mutations were found in the mesenchymal appearing, spindle cell areas of the tumors.

Identical frame shift mutations of the Wnt-1 induced secreted protein 3 (*WISP3*) gene were found in 5 of 27 (18.5%) metaplastic carcinomas. All mutations led to a deletion of a guanine at codon 1141 resulting in a truncated protein known to cause human disease (13, 14). In contrast to *CTNNB1* and *APC* mutations, which were more common in the mesenchymal components of the metaplastic carcinomas, *WISP3* mutations were more frequent in the epithelial cells. Thus, one metaplastic carcinoma (Table 3, case 10) harbored two *WISP3* mutations in both squamous and glandular areas. The remaining four tumors had mutations in the glandular (two cases), squamous (one case), and chondroid (one case) areas. No *AXIN1* or *AXIN2* gene mutations were found in our study. Supplementary Table S1 shows the mutations found when different tumor areas were analyzed.

Table 2. Summary of clinical and pathologic information of the patients with metaplastic carcinoma

Variable	Value
No. patients	36
Median age (range), y	60 (36-87)
Pathologic stage, *n* (%)	
I/II	16 (44.4)
III/IV	6 (16.7)
Unknown	14 (38.9)
Median tumor size (range), cm	3.5 (0.5-10.5)
Lymph nodes, *n* (%)	
Negative	16 (44.4)
Positive	10 (27.8)
Unknown	10 (27.8)
Site of distant metastasis, *n*	
Lung and pleura	3
Vertebrae	1
Brain	1
Predominant metaplastic component, *n* (%)	
Spindle	12 (33.3)
Squamous	16 (44.4)
Chondroid	6 (16.7)
Osseous	2 (5.6)
Histologic grade, *n* (%)	
1	0
2	11 (30.6%)
3	22 (61.1%)
β-Catenin, *n* (%)	
Normal	3 (8.3)
Aberrant	33 (91.7)

Discussion

Hyperactivation of the canonical Wnt/β-catenin pathway, caused by mutations in such components as β-catenin and APC, is one of the most frequent signaling abnormalities in several human cancers including colorectal carcinomas (15), melanomas (16), hepatoblastomas (17), medulloblastomas (18), prostatic carcinomas (19), and uterine and ovarian endometrioid adenocarcinomas (10, 20–22). In breast cancer, however, evidence of comparable mutations is surprisingly lacking (23). In contrast, there is strong evidence, based on immunohistochemical analyses, that the Wnt/β-catenin pathway is activated (23–25). Importantly, aberrant β-catenin expression in breast cancer is associated with poor clinical outcome (23–26). Metaplastic carcinomas have never been analyzed for Wnt pathway gene mutations.

Because breast carcinoma arises from glandular epithelium, it usually exhibits the features of an adenocarcinoma. However, in some cases, part or all of the neoplastic cells differentiate into a nonglandular growth pattern by a process

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termed ''metaplasia,'' which signifies the change of one cell type into another. This characterizes the special type of breast carcinoma termed metaplastic carcinoma. We hypothesized that the phenotypic changes of metaplastic carcinomas in the breast are likely the result of alterations of genes involved in cell fate and differentiation.

The Wnt gene family plays roles in the development of the mammary gland and in breast cancer (23, 27). The consequences of Wnt signaling are often concerned with cell fate determination in several organs including the mammary gland. Interestingly, in animals whose endogenous β-catenin gene was mutated, the predominant effect in the mammary gland was squamous metaplasia, suggesting that high levels of β-catenin can result in a switch from an alveolar to an epidermal cell type (28). Furthermore, Wnt pathway activation has been shown to play a central role in the process of epithelial to mesenchymal transition during development and in breast cancer, whereby glandular epithelial cells undergo a genotypic and phenotypic switch from an epithelial cell to an elongated spindle cell (29, 30). In cancer, there is strong evidence showing that the dynamic process of epithelial to mesenchymal transition is important in tumor invasion and metastasis (31 – 33).

We have pursued our studies on the investigation of the relevance and mechanism of deregulation of Wnt signaling pathway components in 36 histologically verified primary metaplastic carcinomas of the breast. This is, in fact, a substantial number of primary tumors of this particular histologic subtype of breast cancer because only 1% to 5% of breast cancers are metaplastic (3, 34). Evidence of Wnt pathway activation was found in nearly all the primary metaplastic carcinomas. Forty-one percent of the metaplastic carcinomas carried mutations on genes critical in the canonical Wnt pathway, with three tumors containing mutations in two different genes. We identified *CTNNB1* exon 3 mutations in 25.9% of tumors. Mutations inactivating the *APC* gene and truncating mutations of the *WISP3* gene were observed in 7.4% and 18.5% of metaplastic carcinomas, respectively. This is the first study to identify mutations of the *CTNNB1*, *APC*, and *WISP3* genes in primary breast carcinomas. Our data provide mechanistic evidence for the observed deregulation and accumulation of β-catenin in this specific subtype of breast cancer.

A number of studies have shown that mutations of the *CTNNB1* and *APC* genes lead to increased levels of β-catenin and promote tumor development (15). Significantly less is known about WISP3. WISP3 is a secreted cysteine-rich protein

Table 3. Summary of β-catenin immunodetection and sequence analysis of Wnt pathway genes in metaplastic carcinomas of the breast

Case	Age, y	Grade	β-Catenin IH			CTNNB1 mutation			APC mutation			WISP3 mutation			Histologic component harboring the mutation
			N	C	M	Nucleotide	Amino acid	Codon	Nucleotide	Amino acid	Codon	Nucleotide	Amino acid	Codon	
1	46	2	Pos	Pos	Neg				A-T	K-Stop	1317	del G	Frameshift	1141	Spindle (*APC*) and glandular (WISP3)
2	52	2	Neg	Pos	Neg										
3	70	3	Pos	Pos	Neg	TCT-TGT	Ser-Cys	33							Spindle
4	67	2	Pos	Neg	Neg	TCT-TAT	Ser-Tyr	37	A-T	K-Stop	1317				Spindle (*APC*) and glandular (CTNNB1)
5	71	2	Pos	Pos	Neg										
6	58	3	Pos	Pos	Neg	ACA-AGA	Thr-Arg	42							Glandular
7	87	2	Pos	Pos	Neg	TCT-TGT	Ser-Cys	33							Spindle
8	48	3	Pos	Pos	Neg										
9	50	3	Pos	Pos	Neg										
10	71	3	Pos	Pos	Neg							del G	Frameshift	1141	Squamous and glandular
11	40	3	Pos	Pos	Weak										
12	58	3	Neg	Neg	Weak	ACA-AGA	Thr-Arg	42							Glandular
13	33	3	Neg	Neg	Weak										
14	72	3	Neg	Neg	Pos										
15	35	3	Neg	Pos	Pos										
16	50	3	Pos	Pos	Weak										
17	50	3	Pos	Pos	Pos										
18	42	3	Neg	Pos	Pos							del G	Frameshift	1141	Chondroid
19	60	3	Neg	Neg	Weak							del G	Frameshift	1141	Glandular
20	67	3	Neg	Pos	Weak										
21	36	2	Pos	Pos	Neg										
22	48	2	Neg	Pos	Red										
23	40	3	Pos	Pos	Weak										
24	75	3	Neg	Pos	Neg										
25	89	2	Neg	Pos	Weak	TCT-TGT	Ser-Cys	33				del G	Frameshift	1141	Squamous
26	NA	3	Neg	Neg	Neg	TCT-ACT	Ser-Thr	33							
27	54	3	Pos	Neg	Neg										

Abbreviations: N, nucleus, C, cytoplasm; M, membrane. Pos, positive; Neg, negative; Red, reduced; IH, immunohistochemistry.

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that belongs to the CCN family of growth factors that mediate epithelial and stromal cross talks (35–40). WISP3 is also named CCN6. Our laboratory as well as other investigators have shown that deregulation of this protein family can lead to cancer (41–43). Specifically, our group has previously found that WISP3 is down-regulated in the most lethal form of locally advanced breast cancer, inflammatory breast cancer, and in a group of high-stage noninflammatory breast cancer tumors (44). WISP3 inhibits tumor cell motility and invasion *in vitro* and inhibits tumor growth *in vivo* (42, 45–47). *WISP3* mutations have been found in progressive pseudorheumatoid dysplasia (13, 14) and in colorectal carcinomas (11). The presence of *WISP3* mutations in metaplastic carcinomas of the breast is intriguing in light of our previous data showing that stable small interfering RNA knockdown of WISP3 mRNA and protein in human mammary epithelial cells causes an epithelial to mesenchymal transition and triggers motility and invasion with marked inhibition of E-cadherin (47). The effects of WISP3 inhibition on β-catenin expression and function warrant further investigation.

To date, mammary fibromatosis is the only breast tumor in which *CTNNB1* and *APC* mutations are common pathogenetic events (48, 49). These are rare benign tumors with the capacity for local infiltration and recurrence after surgical excision. Abraham et al. (49) found *CTNNB1* and *APC* mutations in 79% of mammary fibromatosis studied. Interestingly, despite the epithelial nature of metaplastic carcinomas and the mesenchymal origin of fibromatosis, they share some histologic features. They both contain elongated tumor cells that form sweeping and interlacing fascicles and infiltrate the adjacent breast parenchyma. This observation, together with the crucial role of the Wnt pathway in cell differentiation and in the process of epithelial to mesenchymal transition, suggests that mutations of *CTNNB1* and *APC* genes may contribute to the specific phenotypic spindle cell morphology and pattern of tissue infiltration that characterizes these tumors.

We found that three metaplastic carcinomas containing *CTNNB1* (two cases) and *WISP3* mutations (one case) had no nuclear or cytoplasmic accumulation of β-catenin protein, but rather a decrease in its membrane expression. Although detection of β-catenin protein in the nucleus and/or cytoplasm is a hallmark of active Wnt signaling (10), reduced or absent membranous β-catenin has also been implicated in Wnt pathway deregulation (26). This idea is further supported by several studies showing that decreased β-catenin membrane expression is associated with poor prognostic factors in breast cancer (26, 50).

In the present study, mutations of *CTNNB1*, *APC*, and *WISP3* genes were identified in 41% of metaplastic carcinomas whereas aberrant β-catenin protein was detected in nearly all the metaplastic carcinomas analyzed. There are several potential explanations for these results. It is possible that the stabilization of β-catenin results from deregulation of other signaling pathways that can regulate β-catenin such as those activated by loss of phosphatase and tensin homologue, activation of the epidermal growth factor receptor family, and by p53 function, which have been shown to be altered in breast cancer (4, 27). Another possible explanation for the aberrant β-catenin protein expression in the absence of identified mutations of *CTNNB1*, *APC*, and *WISP3* genes is that there may be mutations in other portions of these genes that result in proteins with yet undescribed functions, or mutations of other components of the Wnt signaling pathway including Wnt receptors.

In summary, we have shown that β-catenin deregulation is a common feature of metaplastic carcinomas of the breast, and discovered that in 41% of cases the mechanisms for deregulating β-catenin include specific mutations of *CTNNB1* gene itself and mutational inactivation of *APC* or *WISP3* genes. To date, this is the only type of breast cancer with frequent Wnt pathway gene mutations. Furthermore, our data provide a mechanistic explanation for the aberrant nonglandular differentiation characteristic of metaplastic carcinomas and identify a pathway that may be the basis of targeted treatment for this form of breast cancer.

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