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Complete NUB1 depletion in ER-negative breast cancer progression in paired primary–metastatic cases: a case series

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Abstract

Background NEDD8 ultimate buster 1 is an interferon-inducible tumor suppressor increasingly recognized as a prognostic biomarker in breast cancer. Low cytoplasmic expression correlates with aggressive disease, yet little is known about its dynamics between primary and metastatic estrogen-receptor-negative tumors.

Objective The objective of this report is to compare NEDD8 ultimate buster 1 expression in paired estrogen-receptor-negative primary and metastatic breast cancer tissues, clarifying its role in tumor progression.

Case presentation Two postmenopausal Caucasian women, patient A (ID: 14,024, aged 58 years) and patient B (ID: 20,996, aged 54 years), both diagnosed with estrogen-receptor-negative, human epidermal growth factor receptor 2-negative, grade III invasive ductal carcinoma, underwent NEDD8 ultimate buster 1 immunohistochemical analysis on matched primary breast tumors and corresponding metastatic lymph nodes. Both primary tumors exhibited high nuclear but low cytoplasmic NEDD8 ultimate buster 1, shifting to complete loss in metastases. The patient with higher primary cytoplasmic NEDD8 ultimate buster 1 had longer relapse-free and overall survival.

Conclusion Loss of NEDD8 ultimate buster 1 in metastases may signal a transition from hypoxia-driven nuclear localization to unchecked metastatic progression. These findings highlight NEDD8 ultimate buster 1 as a potential prognostic marker and therapeutic stratifier, warranting larger studies to confirm its mechanistic and clinical relevance.

Keywords NUB1, Breast cancer, ER-negative, Tumor progression, Biomarkers, Immunohistochemistry

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Background

NEDD8 ultimate buster 1 (NUB1) is emerging as a potential prognostic biomarker in breast cancer (BC), with reduced expression linked to increased tumor aggressiveness and poorer outcomes [1, 2]. First identified in 2002 as a regulator of the ubiquitin-like protein NEDD8, NUB1 plays a key role in protein modification and degradation pathways [1]. Structurally, it consists of 601 amino acids with a molecular mass of 69.1 kDa [1].

NUB1 is localized in the cytoplasm and nucleus, with its distribution influenced by cellular stressors such as hypoxia [2]. Under hypoxic conditions, NUB1 accumulates in the nucleus, while reoxygenation shifts it to the cytoplasm [3]. Hypoxia, a hallmark of the tumor microenvironment [2, 4], likely modulates NUB1 function during cancer progression (Fig. 1) [2]. Notably, differential NUB1 expression has been observed between primary and metastatic BC tissues, with reduced levels correlating with higher metastatic potential and poorer outcomes [5, 6]. Dysregulation of NUB1 has been implicated in many cancers, including breast cancer and gastric cancer, where its expression is often diminished [2, 7]. In BC, low cytoplasmic NUB1 expression is associated with increased apoptosis and proliferation, indicating aggressive tumor progression [$p < 0.05$; HR 1.779 (1.006–3.346)] [2]. Similarly, in gastric cancer, reduced NUB1 expression is linked to worse prognosis [$p < 0.05$; HR 0.33 (0.20–0.54)] [7], underscoring its role in cancer progression and metastasis.

BC classification relies on biomarkers such as ER, PR, human epidermal growth factor receptor 2 (HER2), and Ki-67 [8]. High Ki-67 is associated with poorer prognosis in triple-negative breast cancer (TNBC) [9], while HER2-positive tumors tend to be more aggressive [10]. These markers guide treatment strategies [11], but NUB1 may offer additional prognostic value independent of traditional markers [7].

Loss of NUB1 expression may drive BC progression, and initial nuclear accumulation under hypoxia gives way to complete depletion, which disrupts neddylation

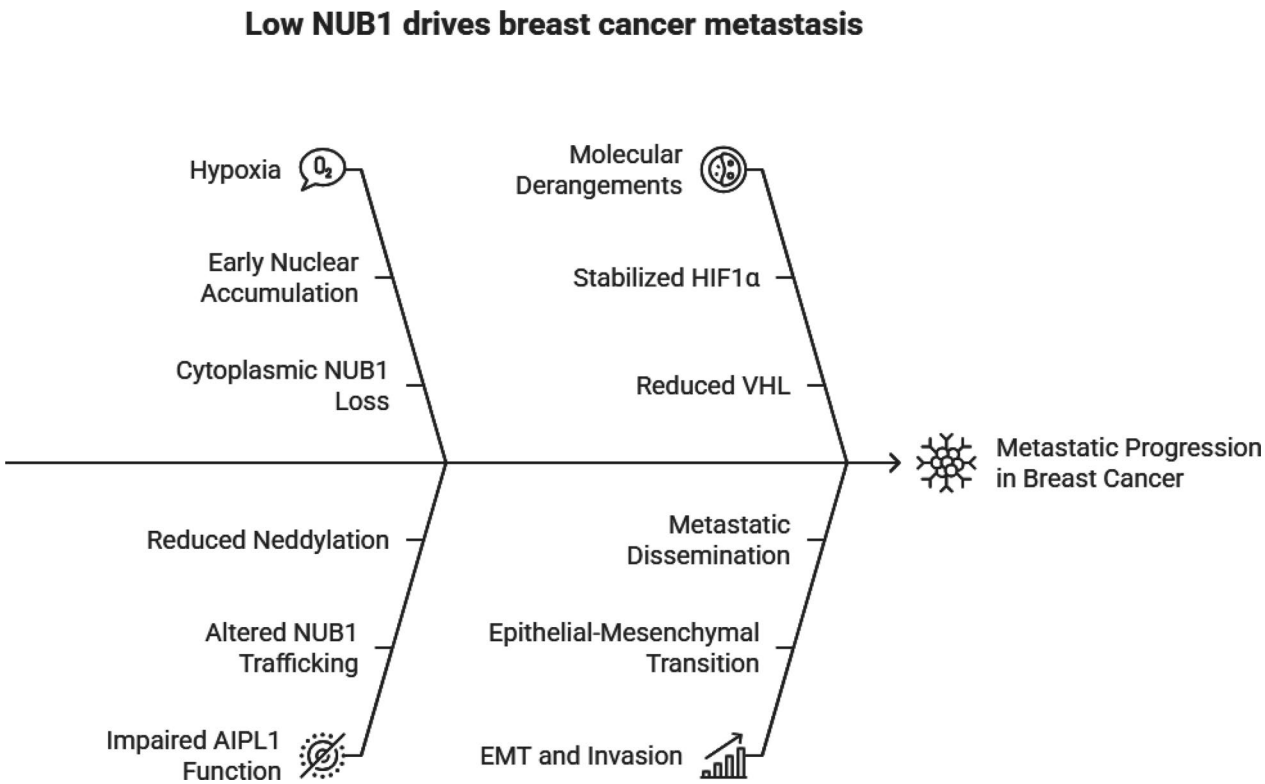

Fig. 1 NEDD8 ultimate buster 1 (NUB1) loss to metastatic progression in breast cancer. Hypoxia and impaired aryl hydrocarbon receptor-interacting protein-like 1 chaperone function alter negative regulator of ubiquitin-like proteins 1 (NEDD8 ultimate buster 1) trafficking, producing early nuclear accumulation under hypoxia, and with disease progression, cytoplasmic NEDD8 ultimate buster 1 loss. Chronic NEDD8 ultimate buster 1 depletion perturbs neddylation (reduced neddylated Cullin-1 and neural precursor cell expressed, developmentally downregulated protein 8), stabilizes hypoxia-inducible factor 1-alpha, reduces von Hippel–Lindau tumor suppressor, and promotes accumulation of cyclin-dependent kinase inhibitor 1A (p21) and cyclin-dependent kinase inhibitor 1B (p27). These molecular derangements converge to promote epithelial–mesenchymal transition, invasion, and metastatic dissemination, consistent with the poorer survival observed in estrogen-receptor-negative patients with low cytoplasmic NUB1

control and promotes EMT, invasion, and metastatic spread (Fig. 1). [2]. Examining NUB1 expression in both primary and metastatic tumors from the same patients is critical for understanding tumor evolution and metastatic behavior [12]. Primary and metastatic tumors often exhibit distinct molecular profiles due to genetic and epigenetic changes during progression [2]. Comparing NUB1 expression in these sites helps determine whether NUB1 loss is an early event in tumorigenesis or a later adaptation during metastasis. For example, consistently low NUB1 expression in both primary and metastatic tumors may indicate an inherently aggressive phenotype, while a decline from primary to metastatic sites could suggest its role in facilitating metastatic spread. Such insights can guide therapeutic decisions, such as prioritizing systemic therapies for NUB1-negative metastases or surgical intervention for NUB1-positive lesions.

Clinically, incorporating NUB1 as a biomarker could enable targeted therapeutic strategies, focusing on high-risk sites while avoiding overtreatment of less aggressive lesions. This study analyses NUB1 expression in primary and metastatic tumors of two patients with BC via immunohistochemistry (IHC), proposing that differential NUB1 expression at various disease stages may offer insights into tumor evolution and facilitate personalized oncological management.

Case presentation

This case report includes two Caucasian female patients with BC who succumbed to death due to the metastatic dissemination of primary BC cells to the lymphatic system. Patient A (14,024) was 58 years old, whereas patient B (20,996) was 54 years old. These patients with BC were identified as postmenopausal, with breast tumor sizes of 5.5 cm and 2.4 cm, respectively. These patients tested negative for both ER and HER2. Multiple pathological lymph nodes were observed in patient samples. These patients had a history of grade III (Nottingham modified Bloom–Richardson System score) infiltrating ductal carcinoma (IDC). No systemic anticancer therapy was recorded for either patient in the clinical archive; therefore no treatment interventions were performed prior to metastatic progression.

The commercialized NUB1 protein antibody was first validated using IHC. Diaminobenzidine (DAB) detection was performed on tissue microarray (TMA) sections. The DAKO Envision Plus system (Autostainer Link 48, Agilent) was used to observe formalin-fixed paraffin-embedded (FFPE) tissues. Brown staining indicated immunoreactivity (Fig. 2).

Two pathologists scored NUB1 expression as an intensity percentage score (IPS) in the nucleus and cytoplasm of the primary and metastatic tissues. Both patients had low NUB1 cytoplasmic localization and high NUB1 nuclear localization in the primary tumor, while metastatic tissues showed no NUB1 expression in the nucleus and cytoplasm. (Fig. 2).

Upon survival monitoring at the primary biopsy, patient A (14,024) remained relapse-free for 532 days with a 1 + (IPS 100) score of cytoplasmic NUB1 and a 3 + (IPS: 300) score of nuclear NUB1 (IPS cytoplasm/nucleus ratio 100/300). Further, patient B (20,996) remained relapse-free for 394 days after diagnosis, with a slightly low 1 + (IPS: 50) score of cytoplasmic NUB1 and 3 + (IPS: 300) score of nuclear NUB1 (IPS cytoplasm/nucleus ratio 50/300) (Table 1).

In metastatic tissues, different results were obtained: patient A (14,024) with a 0 (IPS 0) score for cytoplasmic NUB1 and 0 (IPS: 0) score for nuclear NUB1 (IPS cytoplasm/nucleus ratio 0/0), while patient B (20,996) with a 0 (IPS: 0) score for cytoplasmic NUB1 and 0 (IPS: 0) score for nucleus NUB1 (IPS cytoplasm/nucleus ratio 0/0) (Table 1). Metastatic BC tissues of both patients showed no NUB1 expression. Upon comparing IHC scores with survival probability, the overall survival for patient A was reported as 1260 days, whereas for patient B, it was reported as only 612 days after diagnosis (Table 1). Both tumors showed low cytoplasmic to high nuclear NUB1 positive staining in primary tissues, with no NUB1 expression in metastatic tissues. DAB staining observed in metastatic sites likely reflects background staining or nontumor tissue elements rather than true NUB1 expression. Patient A had relatively high NUB1protein expression 1 + (IPS 100) score of cytoplasmic NUB1 and a 3 + (IPS: 300) score of nuclear NUB1 (IPS cytoplasm/nucleus ratio 100/300) and thus had better overall survival. Patient B had a relatively low expression 1 + (IPS: 50) score of cytoplasmic NUB1 and 3 + (IPS: 300) score of nuclear NUB1 (IPS cytoplasm/nucleus ratio 50/300) and thus poorer overall survival. NUB1 expression differs in primary and metastatic BC tissues.

Discussion

This study evaluated NEDD8 ultimate buster 1 (NUB1) protein expression in matched primary and metastatic ER-negative breast cancer (BC) tissues from two postmenopausal patients, revealing distinct subcellular localization patterns with potential biological and clinical significance. In both cases, immunohistochemistry demonstrated low cytoplasmic but high nuclear NUB1 expression in primary tumors, with complete loss in metastatic lesions. This rare paired primary–metastatic ER-negative dataset offers a unique window into dynamic NUB1 regulation during tumor evolution, suggesting

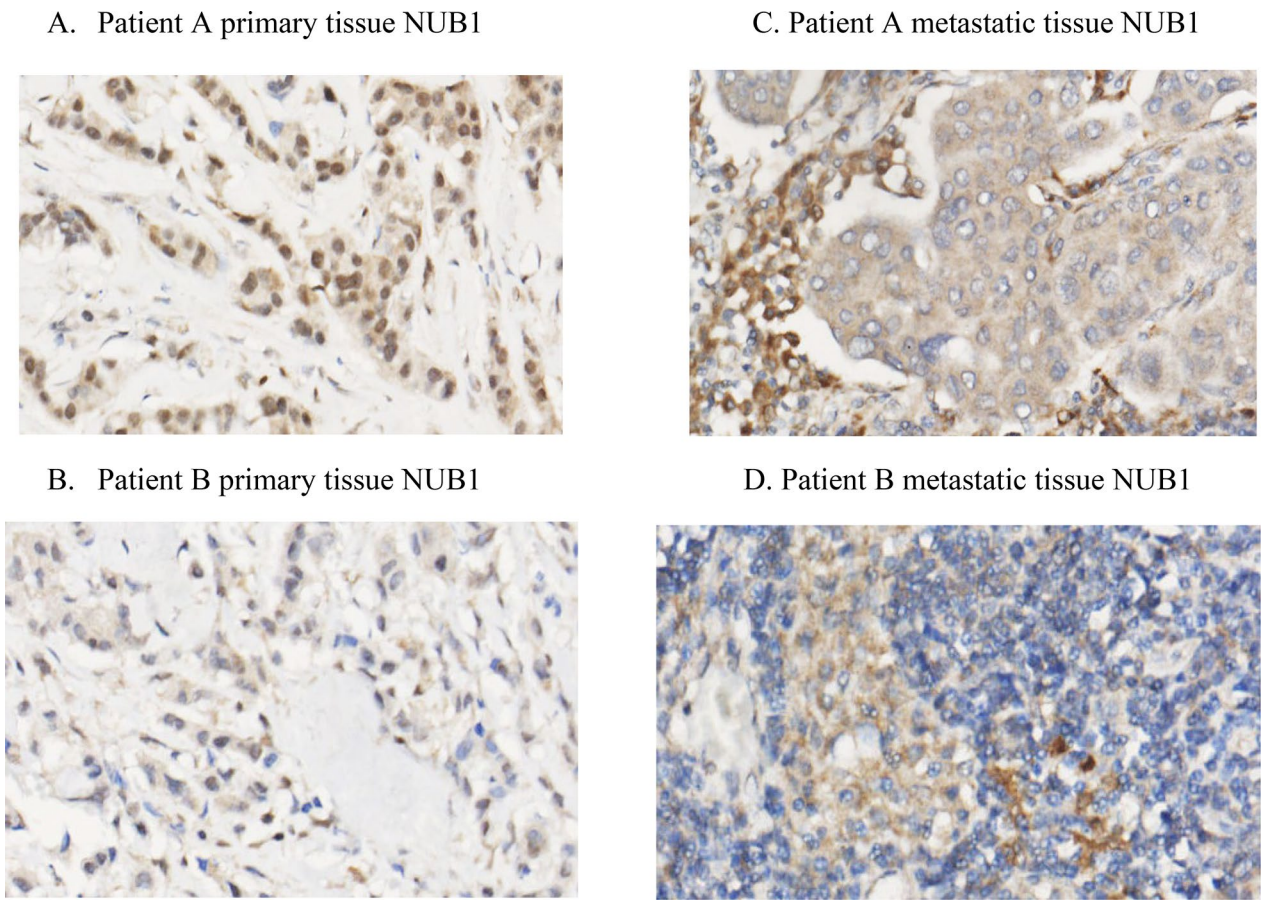

Fig. 2 Immunohistochemistry staining of primary and metastasis tissues of patients with breast cancer. The representative images of immunohistochemical analysis show NEDD8 ultimate buster 1 (NUB1) protein localizations in breast cancer tissues. Brown staining with diaminobenzidine indicates immunoreactivity. All magnification of the images is 40×

Table 1 Summary of IHC analysis of NUB1 protein expression in tissues at the primary and metastatic biopsies in patients with BC and its relevance to their survival status

Patient: ID	IHC													
	Primary-NUB1-C			Primary-NUB1-N			Metastasis-NUB1-C			Metastasis-NUB1-N			Survival status	
	I	%	IPS	I	%	IPS	I	%	IPS	I	%	IPS	RFS	OS
Patient A (14,024)	1 +	100	100	3 +	300	300	0	0	0	0	0	0	532	1260
Patient B (20,996)	1 +	50	50	3 +	300	300	0	0	0	0	0	0	394	612

IHC immunohistochemistry, *0* no staining, *1* + mild staining, *3* + strong staining, *C* cytoplasm, *N* nucleus, *I* intensity, *%* percentage, *IPS* intensity percentage score, *RFS* relapse-free survival, *OS* overall survival

that its depletion may be a critical molecular event in metastatic progression, particularly in aggressive, hormone receptor-negative subtypes.

Our findings align with prior reports linking low cytoplasmic NUB1 to poorer prognosis in ER-negative BC [2] and in gastric cancer [7], where diminished NUB1 is associated with increased invasion, proliferation, and epithelial–mesenchymal transition (EMT). The high nuclear NUB1 seen in primary tumors is consistent with evidence that hypoxia can drive nuclear accumulation [2, 13], likely reflecting an adaptive tumor-suppressive response to cellular stress, aiding proteostasis and cell-cycle regulation. In contrast, complete absence of NUB1 in metastases implies a late-stage collapse of

this protective mechanism, potentially mediated by genetic and epigenetic alterations, disrupted signaling pathways, and deregulation of ubiquitin–proteasome or neddylation processes, all of which could promote EMT and metastatic dissemination [12].

From an evolutionary standpoint, these results may represent two distinct phases in BC progression. Initially, hypoxia-induced nuclear localization of NUB1 may constrain malignant behavior, but with disease advancement, loss of NUB1 removes these safeguards, enabling clonal expansion of aggressive tumor cells and immune evasion [7, 15]. This dual-phase behavior underscores NUB1's potential role as both a stress-response mediator and a gatekeeper against metastatic spread.

The recent study on NUB1 [14] extends this perspective by demonstrating that its expression may also predict response to 5-Fluorouracil, epirubicin, cyclophosphamide (FEC) chemotherapy in ER-positive BC. Although our cases were ER-negative, the convergence of prognostic and predictive roles across subtypes suggests that NUB1 may act through shared biological pathways, albeit modulated by distinct hormonal and molecular contexts. This supports a rationale for evaluating NUB1 as a universal biomarker of progression, independent of receptor status.

Clinically, integrating NUB1 status with residual tumor cellularity (RTC) following neoadjuvant chemotherapy (NAC) could enhance prognostic precision. Achieving pathological complete response (pCR) or maintaining RTC below 40% is linked to improved survival outcomes [16, 17]. A combined NUB1–RTC assessment could identify high-risk patients, particularly those with NUB1-negative metastases, who might benefit from intensified adjuvant therapy, targeted agents, or closer surveillance. In parallel, strategies to restore or mimic NUB1 function could offer novel therapeutic avenues.

Therapeutically, our findings highlight the potential of targeting the NUB1 pathway—whether by restoring or stabilizing NUB1, modulating its chaperone AIPL1 [2], or carefully leveraging neddylation inhibitors such as MLN4924 [18]. While these strategies remain hypothesis generating, they offer a foundation for preclinical exploration of NUB1-directed interventions in metastatic breast cancer [2].

This study is limited by its small sample size, making these observations hypothesis generating rather than definitive. Potential confounders, inter-patient variability, and treatment history may have influenced NUB1 expression patterns. Future research should include larger, molecularly diverse cohorts with longitudinal sampling to track NUB1 changes from diagnosis to recurrence, complemented by genomic and transcriptomic profiling to uncover regulatory mechanisms. Functional studies could further test whether reintroducing NUB1 in metastatic cells reverses aggressive phenotypes.

Taken together, our results position NUB1 as both a marker and a potential regulator of metastatic transition in BC, with implications for risk stratification and therapeutic targeting. By combining molecular profiling of NUB1 with established clinicopathologic predictors, it may be possible to move toward more precise, biology-driven management strategies that address the fundamental processes underpinning tumor evolution.

Conclusion

NUB1 expression shifts from nuclear predominance in primary ER-negative breast tumors to complete loss in metastases, reflecting a transition from an adaptive, stress-response phase to a permissive state that facilitates tumor evolution, progression, and poor prognosis. These findings, together with emerging evidence of NUB1's predictive role in chemotherapy response, support its potential as both a prognostic biomarker and a therapeutic target. Larger, molecularly diverse studies are warranted to validate its clinical utility and to guide strategies aimed at restoring or mimicking its tumor-suppressive functions.

Abbreviations

BC	Breast cancer
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
IDC	Invasive ductal carcinoma
NUB1	NEDD8 ultimate buster 1
IHC	Immunohistochemistry
IPS	Intensity percentage score
EMT	Epithelial–mesenchymal transition
pCR	Pathologic complete response
RTC	Residual tumor cellularity
NAC	Neoadjuvant chemotherapy
FEC	5-Fluorouracil, epirubicin, cyclophosphamide
HIF-1α	Hypoxia-inducible factor 1, alpha subunit
VHL	Von Hippel–Lindau (tumor suppressor)
AIPL1	Aryl hydrocarbon receptor-interacting protein-like 1

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Author contributions

Conceptualization: KT and SC. Methodology: KT and FP. Software: FP. Validation: GT and FI. Formal analysis: FP, GT, and FI. Investigation: AA, MS, FP, and AY. Resources: KT, GT, and SC. Data curation: MA, FI, and MS. Writing—original draft preparation: MA and KT. Writing—review and editing: GT, SC, and MA. Visualization: MS and AA. Supervision: KT, FI, and AA. Project administration: FP and AY. Funding acquisition: KT.

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Data availability
All data are incorporated into the article. Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
The institutional review board of the University of Oxford approved the study protocol (C02.216, "The pathobiology of neoplasia in human tissues").

Consent for publication
Written informed consent was obtained from the patients for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

Maria Arshad is the first author and holds a PhD in Medical Sciences from Universiti Sains Islam Malaysia, Her doctoral research investigated NUB1 protein as a predictive biomarker in FEC-treated breast cancer, using advanced immunohistochemistry and molecular biology methods.

Ka-Liong Tan corresponding author, is a senior lecturer in pharmacology at the Faculty of Medicine & Health Sciences, Universiti Sains Islam Malaysia, and the principal investigator of this study. He has extensive expertise in pharmacology, molecular oncology, and biomarker discovery.