High mitochondrial content is associated with breast cancer aggressiveness

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Abstract. Mitochondria are relevant for cancer initiation and progression. Antibodies against mitochondrially encoded cytochrome *c* oxidase II (MTCO2), targeting a mitochondria specific epitope, can be used to quantitate the mitochondria content of tumor cells. The present study evaluated the impact of the cellular mitochondrial content on the prognosis of patients with breast cancer using immunohistochemical analysis on 2,197 arrayed breast cancer specimens. Results were compared with histological tumor parameters, patient overall survival, tumor cell proliferation using Ki67 labeling index (Ki67LI) and various other molecular features. Tumor cells exhibited stronger MTCO2 expression than normal breast epithelial cells. MTCO2 immunostaining was largely absent in normal breast epithelium, but was observed in 71.9% of 1,797 analyzable cancer specimens, including 34.6% tumors with weak expression, 22.3% with moderate expression and 15.0% with strong expression. High MTCO2 expression was significantly associated with advanced tumor stage, high Bloom-Richardson-Elston/Nottingham (BRE) grade, nodal metastasis and shorter overall survival (P<0.0001 each). In multivariate analysis, MTCO2 expression did not provide prognostic information independent of BRE grade, pathological tumor and pathological lymph node status. Additionally, significant associations were observed for high MTCO2 expression and various molecular features, including high Ki67LI, amplifications of *HER2*, *MYC*, *CCND1* and *MDM2*, deletions of *PTEN*, 8p21 and 9p, low estrogen receptor expression (P<0.0001 each) and progesterone receptor expression (P<0.0001). The present study demonstrated that high MTCO2 expression was strongly associated with a poor prognosis and unfavorable phenotypical and molecular tumor features in patients with breast cancer. This suggests that the mitochondrial content may have a pivotal role in breast cancer progression.

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Abbreviations: IHC, immunohistochemistry; Ki67LI, Ki67 labeling index; TMA, tissue microarray; MTCO2, mitochondrially encoded cytochrome *c* oxidase II

Key words: MTCO2, IHC, TMA, prognosis, breast cancer

Introduction

Breast cancer, the most common malignancy in women (1), is treated by surgical removal of the cancer. In addition, adjuvant systemic therapy is given depending on the perceived aggressiveness of the removed cancer. Currently the established prognostic parameter include histological grade, tumor size, presence of lymph node metastasis, tumor cell proliferation (Ki67 labeling index; Ki67LI) as well as hormonal receptor and HER2 status (2-4) (Ki67) (5). In many patients, supplementary molecular parameters are analyzed (6-8). These molecular classifiers are built on multiplexed analysis of the mRNAs of 21-70 genes (9-11).

The rising interest in mitochondrial function and dysfunction on cancer development has been reviewed by Davis and Williams and Hsu *et al* (12,13). The loss of proliferation control in cancer cells may result in cellular

masses that extend beyond the capacity of the supporting vasculature, leading to oxygen and nutrient deprivation. Hence, tumor cells must adapt to overcome these restrictions. Mitochondria are key organelles for energy production in normal and neoplastic cells. Quantity and activity of mitochondria are essential for tumor growth (reviewed in refs. 12-16). Mutations in mitochondrial genes or aberrant mitochondrial content have been described to occur in various cancer types (17-20). An increased mitochondria quantity has earlier been linked to aggressive tumor phenotype and poor prognosis in lung (21), colorectal (22,23), prostate (24), gastric (25), cervical (18), and ovarian cancer (26). In glioma, however, high mitochondria content was linked to favorable prognosis (27). In one study, on 76 breast carcinomas, a prognostic impact of the mitochondria count was also suggested (28). Focused on these reports, we assumed that the cellular mitochondria content of breast cancer cells might potentially be clinically relevant in breast cancer.

The mitochondrially encoded cytochrome *c* oxidase II (MTCO2) monoclonal antibody recognizes a 60 kDa non-glycosylated protein subunit of cytochrome *c* oxidase in mitochondria found in human cells and has been used to reveal the mitochondrial content of tumor cells in previous studies (24,28,29). We tested the clinical relevance of the cellular mitochondria content in breast cancer on a pre-existing breast cancer tissue microarray (TMA) containing more than 2,000 cancers. The data show that a 'mitochondrion-rich phenotype' represents a strong and independent predictor of patient prognosis in breast cancer.

Materials and methods

Patients. A total of 2,197 human breast cancer samples from paraffin-embedded tissue specimens fixed in 4% neutral buffered formalin were used (30). The breast cancer samples were consecutively collected between 1984 and 2000 and follow-up data were retrospectively collected. The median patient's age was 63 (range, 25-101) years. Overall survival data were available from 1,982 patients (713 patients with and 1,508 without event). The mean follow-up time was 63 months (range, 1-176 months). The TMA was produced as detailed earlier in (31). In short, from each patient one 0.6 mm core was taken from a representative cancer tissue block. All tissues were distributed among 6 TMA blocks, each containing 263-522 tumor samples. Four-micrometre sections of the TMA blocks were transferred to an adhesive coated slide system (Instrumedics Inc.) for immunohistochemistry (IHC) analysis. Molecular data used in this study were available from previously published studies. These included amplification/deletion data obtained by fluorescence *in situ* hybridization for *HER2*, *MYC*, 8p21, 9p21, and *PTEN*, as well as Ki67LI (30,32-34).

IHC. Freshly cut TMA sections were processed the same day. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min at 121˚C in pH 7.8 Tris-EDTA-Citrate buffer prior to incubation with the mouse monoclonal antibody MTCO2 (Abcam; #ab3298; 1/450 dilution). Bound antibody was visualized using the EnVision kit (Dako). MTCO2 staining was homogenous in the analyzed tissue samples and staining intensity of all cases was semiquantitatively assessed in four categories: Negative (no visible staining), weak (1+ staining intensity), moderate (2+ stainong intensity) and strong (3+ staining intensity).

Statistical analysis. Contingency tables were calculated to study associations between MTCO2 expression and clinicopathological variables, and the chi-square (likelihood) test was used to find significant relationships. Analysis of variance and F-test was applied to find associations between MTCO2 staining levels and tumor cell proliferation as measured by the Ki67LI. Kaplan-Meier curves were generated using overall survival as the clinical endpoint. The log-rank test was applied to test the significance of differences between stratified survival functions. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological and molecular variables. JMP 12.0 software (SAS Institute Inc.) was used.

Results

Technical issues. A total of 1,797 (81.8%) of the 2,197 arrayed tumor samples were interpretable in our TMA analysis. Non-informative cases (400 spots; 18.2%) were due to missing tissue samples or the absence of unequivocal cancer tissue in the TMA spot.

MTCO2 immunostaining in normal breast tissue and breast cancer. There were 20 normal breast tissue samples included in our TMA. Normal breast tissues showed negative to moderate MTCO2 staining in luminal cells under the chosen experimental conditions. In cancer, MTCO2 immunostaining was considered weak in 34.6%, moderate in 22.3% and strong in 15.0% of tumors. A total of 506 (28.2%) showed no detectable MTCO2 staining and were categorized as negative. Characteristic images of MTCO2 immunostainings are shown in Fig. 1. The intensity of MTCO2 immunostaining varied between histological breast cancer subtypes (Table I). Strong MTCO2 staining was significantly more common in medullary (27.9%), papillary (16.0%) and cancers of no special type (NST; 16.6%) than in lobular (6.9%) or tubular carcinomas (4.9%). Strong MTCO2 staining was also commonly seen in some of the rare breast cancer subtypes such as in 3 of 13 carcinomas with apocrine differentiation, 17 of 61 carcinomas with medullary features and 2 of 12 glycogen-rich clear cell type carcinomas (Table SI).

Association with tumor phenotype and molecular features. High levels of MTCO2 immunostaining were significantly associated with high pT stage, high BRE grade, estrogen and progesterone receptor negativity as well as HER2 overexpression or amplification ($P<0.0001$ each, Tables I and II). This was also seen for NST carcinomas ($P\leq0.01$, Table I). Further analyses with previously described frequent and prognostic relevant molecular features of breast cancers such as HER2 (35), and c-MYC- amplification (32) as well as deletions of 8p21 (34), 9p21 (33), and 10q23 (36) showed

Table I. Association between MTCO2 staining and breast cancer phenotype.

Characteristics	N	MTCO2 staining				P-value
		Negative, %	Weak, %	Moderate, %	Strong, %	
All cases	1,797	28.2	34.6	22.3	15.0	
Histology						
NST	1,281	24.2	36.0	23.2	16.6	
Lobular carcinoma	233	46.4	33.1	13.7	6.9	<0.0001[a]
Medullary carcinoma	61	18.0	26.2	27.9	27.9	0.0761[a]
Cribriform carcinoma	55	34.6	27.3	21.8	16.4	0.3461[a]
Mucinous carcinoma	51	45.1	33.3	19.6	2.0	0.0005[a]
Tubular carcinoma	41	48.8	39.0	7.3	4.9	0.0004[a]
Papillary carcinoma	25	20.0	28.0	36.0	16.0	0.5425[a]
Apocrine carcinoma	13	23.1	15.4	38.5	23.1	0.3460[a]
Other rare types[b]	22	9.1	27.3	50.0	13.6	0.0399[a]
pT stage						
pT1	631	36.3	40.3	17.6	5.9	<0.0001 (<0.0001[c])
pT2	851	24.2	32.1	24.7	19.0	
pT3	98	25.5	30.6	24.5	19.4	
pT4	209	21.1	30.1	24.9	23.9	
BRE grade						
G1	423	41.6	37.8	13.5	7.1	<0.0001 (<0.0001[c])
G2	673	29.9	35.5	23.5	11.1	
G3	564	15.4	26.6	29.6	28.4	
Nodal stage						
pN0	761	33.5	34.2	22.7	9.6	<0.0001 (0.0063[c])
pN1	644	25.6	36.2	20.3	17.9	
pN2	103	18.5	38.8	24.3	18.5	
pN3	0	0.0	0.0	0.0	0.0	
Estrogen receptor						
Negative	406	16.01	29.31	28.82	25.86	<0.0001 (<0.0001[c])
Positive	1,296	31.33	36.5	20.45	11.73	
Progesterone receptor						
Negative	1,059	29.27	32.01	22.95	15.77	<0.0001 (0.0795[c])
Positive	569	26.19	40.07	19.68	14.06	

[a]vs. NST; [b]Other types included adenoid-cystic carcinoma, carcinoma with apocrine differentiation, carcinoma with medullary features, carcinoma with neuroendocrine features, carcinoma with signet-ring-cell differentiation, glycogen-rich clear cell carcinoma, metaplastic carcinoma of NST and lipid-rich carcinoma; [c]in NST only. Numbers do not always add up to 1,797 in the different categories due to cases with missing data. NST, no special type; pT, pathological tumor; pN, pathological lymph node; MTCO2, mitochondrially encoded cytochrome c oxidase II; BRE, Bloom-Richardson-Elston/Nottingham system.

significant associations with high MTCO2 staining intensity (Table II).

Association with tumor cell proliferation. Data on tumor cell proliferation as evaluated by the Ki67LI were available from a previous study with the same TMA (30). The mean Ki67LI increased from 19.62±0.66 in MTCO2 negative cancers to 37.75±0.93 in cancers with strong MTCO2 staining (P<0.0001). This statistically significant relationship was also seen in tumor subsets with identical pT or pN stage, lobular and medullary carcinoma, BRE grade and *HER2* status as well as 8p and *PTEN* deletion. All data are summarized in Table III.

Prognostic significance of MTCO2 expression. Survival data were available for 1,806 cancers with interpretable IHC results. The rate of surviving patients continuously decreased with increasing levels of MTCO2 immunostaining (P=0.0001; Fig. 2). The association between strong MTCO2 immunostaining and poor prognosis was also seen in the subgroup of NST cancers (P<0.0001; Fig. 2) and in the nodal positive subset (P<0.0001; Fig. 2) and to a much lesser extent

Table II. Association between MTCO2 staining and molecular alterations.

		MTCO2 staining				
Molecular alterations	N	Negative, %	Weak, %	Moderate, %	Strong, %	P-value
HER2 normal	1,141	29.2	36.3	21.2	13.3	<0.0001
HER2 amplified	239	15.5	32.6	30.1	21.8	
MYC normal	1,232	26.9	34.9	23.1	15.1	<0.0001
MYC amplified	64	7.8	29.7	28.1	34.4	
8p21 normal	578	27.7	39.1	20.9	12.3	<0.0001
8p21 deletion	553	17.0	27.7	29.5	25.9	
9p21 normal	835	25.0	33.1	24.4	17.5	0.0182
9p21 deletion	150	16.7	28.0	32.0	23.3	
10q23 normal	904	25.0	35.0	22.9	17.1	<0.0001
10q23 deletion	216	11.6	26.4	36.1	25.9	

MTCO2, mitochondrially encoded cytochrome *c* oxidase II.

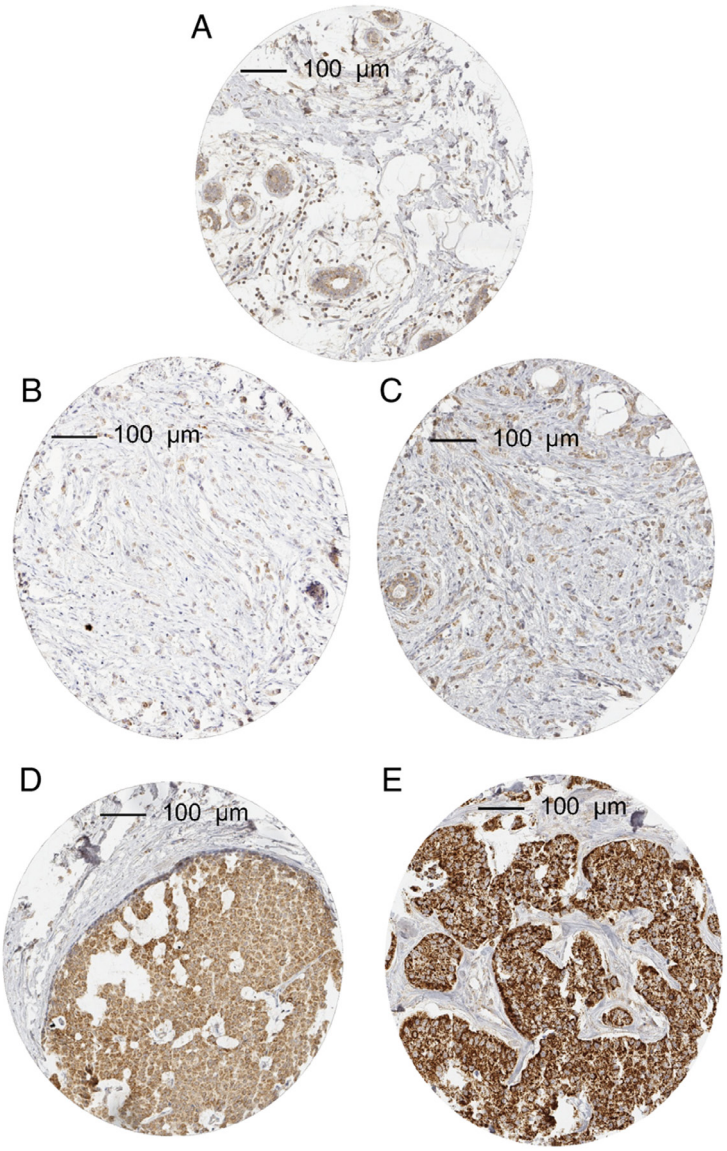

Figure 1. Representative images of MTCO2 staining in breast cancer tissues. (A) Normal breast tissue, (B) negative staining in breast cancer tissue, (C) weak staining in breast cancer tissue, (D) moderate staining in breast cancer tissue and (E) strong staining in breast cancer tissue. Scale bar, 100 μm. MTCO2, mitochondrially encoded cytochrome *c* oxidase II.

Table III. Association between MTCO2 staining and Ki67LI.

Cases	MTCO2 staining	N	Ki67LI	P-value
All cases	Negative	428	19.6±0.7	<0.0001
	Weak	523	27.0±0.6	
	Moderate	338	33.0±0.8	
	Strong	216	37.8±0.9	
No special type	Negative	264	20.7±0.8	<0.0001
	Weak	383	27.8±0.7	
	Moderate	253	33.3±0.9	
	Strong	168	38.0±1.1	
Lobular cancer	Negative	92	16.2±1.2	<0.0001
	Weak	66	20.3±1.4	
	Moderate	24	28.4±2.3	
	Strong	15	26.9±2.9	
Medullary cancer	Negative	9	29.9±5.2	0.0109
	Weak	15	43.7±4.1	
	Moderate	16	50.2±3.9	
	Strong	15	50.9±4.0	
HER2 amplified	Negative	32	26.7±2.3	<0.0001
	Weak	67	34.2±1.6	
	Moderate	64	40.3±1.6	
	Strong	43	41.3±1.9	
MYC amplified	Negative	4	28.5±7.4	0.3927
	Weak	19	38.3±3.4	
	Moderate	17	41.6±3.6	
	Strong	21	41.6±3.2	
8p deletion	Negative	86	24.8±1.5	<0.0001
	Weak	135	30.2±1.2	
	Moderate	145	35.3±1.2	
	Strong	116	40.3±1.3	
PTEN deletion	Negative	24	30.6±3.2	0.0118
	Weak	55	37.7±2.1	
	Moderate	75	41.7±1.8	
	Strong	44	42.2±2.3	
pT1	Negative	192	19.0±0.9	<0.0001
	Weak	200	23.8±0.9	
	Moderate	90	29.9±1.3	
	Strong	31	37.8±2.3	
pT2	Negative	170	19.9±1.1	<0.0001
	Weak	238	29.6±0.9	
	Moderate	179	35.3±1.1	
	Strong	127	37.9±1.3	
pT3	Negative	23	18.2±3.1	<0.0001
	Weak	27	31.2±2.9	
	Moderate	22	30.1±3.2	
	Strong	16	43.8±3.7	
pT4	Negative	41	21.9±2.2	<0.0001
	Weak	57	25.3±1.7	
	Moderate	44	31.9±1.9	
	Strong	41	34.9±2.1	
BRE G1	Negative	150	15.5±0.8	<0.0001
	Weak	127	19.5±0.9	
	Moderate	45	21.4±1.5	
	Strong	25	26.4±1.9	
BRE G2	Negative	170	18.8±0.9	<0.0001
	Weak	208	23.7±0.8	
	Moderate	134	28.9±01.0	
	Strong	63	31.4±1.4	

Table III. Continued.

Cases	MTCO2 staining	N	Ki67LI	P-value
BRE G3	Negative	76	29.4±1.7	<0.0001
	Weak	130	37.5±1.3	
	Moderate	145	40.4±1.2	
	Strong	124	43.2±1.3	
pN0	Negative	219	19.5±0.9	<0.0001
	Weak	216	26.5±0.9	
	Moderate	144	34.5±1.1	
	Strong	59	38.9±1.8	
pN1	Negative	138	19.4±1.2	<0.0001
	Weak	198	27.3±1.0	
	Moderate	111	32.2±1.3	
	Strong	92	39.1±1.4	
pN2	Negative	18	25.6±3.2	0.0064
	Weak	34	29.4±2.3	
	Moderate	23	33.4±2.8	
	Strong	16	41.4±3.4	

Ki67LI, Ki67 labeling index; pT, pathological tumor; pN, pathological lymph node; MTCO2, mitochondrially encoded cytochrome *c* oxidase II; G, grade; BRE, Bloom-Richardson-Elston/Nottingham system.

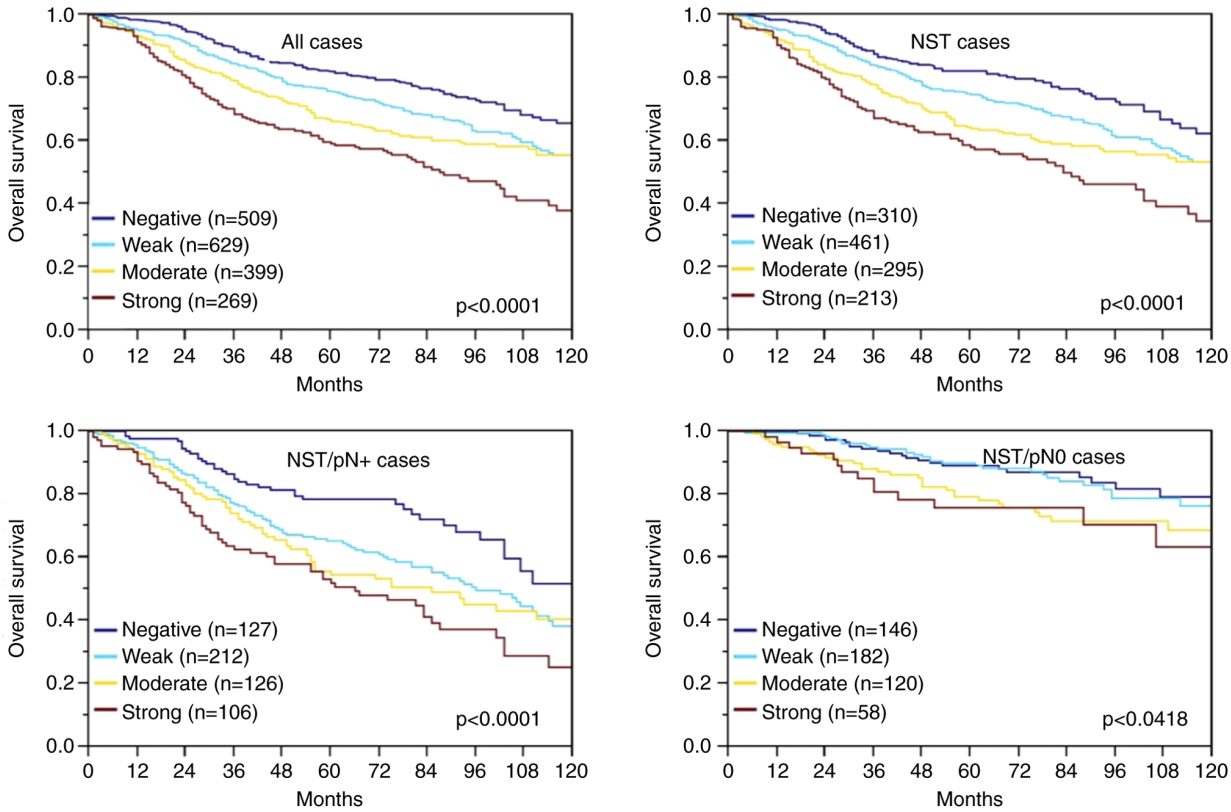

Figure 2. Association between MTCO2 staining and overall survival. MTCO2, mitochondrially encoded cytochrome c oxidase II; NST, no special type; pN, pathological lymph node.

also in nodal negative NST cancers (P=0.0418; Fig. 2). Multivariate analysis for NST cancers including pT stage, nodal status, and BRE grade did not identify MTCO2 immunostaining as an independent prognosticator of survival, however (Table IV).

Discussion

Our study shows that high mitochondria content is significantly linked to disadvantageous tumor phenotype and bad prognosis in breast cancer.

Table IV. Multivariate analysis in all breast cancer cases (n=1,377).

Characteristics	Hazard ratio	P-value	Overall P-value
pT stage			
2 vs. 1	1.52	0.0010	<0.0001
3 vs. 2	1.05	0.7700	
4 vs. 2	1.65	0.0006	
4 vs. 3	1.56	0.2230	
BRE grade			<0.0001
G2 vs. G1	1.35	0.0522	
G3 vs. G1	2.81	<0.0001	
G3 vs. G2	2.08	<0.0001	
pN			<0.0001
1 vs. 0	2.26	<0.0001	
2 vs. 1	2.33	<0.0001	
2 vs. 0	5.27	<0.0001	
MTCO2 staining			0.1464
Weak vs. negative	1.33	0.0396	
Moderate vs. weak	1.01	0.9109	
Strong vs. moderate	0.88	0.4133	

pT, pathological tumor; pN, pathological lymph node; MTCO2, mitochondrially encoded cytochrome *c* oxidase II; G, grade; BRE, Bloom-Richardson-Elston/Nottingham system.

MTCO2 immunostaining is highly specific for the mitochondrial DNA encoded second subunit of cytochrome *c* oxidase and can thus be used to quantitate the mitochondria content by IHC (29). Although mitochondria are present in every normal and neoplastic human cell, 28.2% of our tumors had a negative staining result. This was due to our approach to define experimental conditions, which distinguish cancers with low and high mitochondria quantities. The higher level of MTCO2 immunostaining in breast cancers as compared to normal breast tissues fits with the concept that neoplastic transformation goes along with higher cellular activity requiring more active mitochondria. That a striking further increase of MTCO2 immunostaining was detected with rising tumor grade and stage, demonstrates that elevated numbers of mitochondria are also supporting cancer progression. This is consistent with increasing energy requirement and a rearranged metabolism during tumor progression. Our data fit well with findings in multiple other cancer types, including lung (21), colorectal (22,23), prostate (24), gastric (25), cervical (18), and ovarian cancer (26), where a similar link between high levels of MTCO2 with adverse tumor phenotype and bad prognosis was shown.

In this study, a ubiquitously expressed protein was quantitated by IHC. The TMA approach is optimal for the identification of subtle staining differences of proteins that are abundantly present in cancer, such as mitochondrial components, because TMAs enable maximal experimental standardization at all levels. In our study, more than 1,700 breast cancers were analyzed the same day for maximal standardization. Moreover, all TMA sections were cut on one day immediately before staining in order to avoid unequal decay of a tissues reactivity to antibody binding (37). Finally, one pathologist interpreted all immunostainings in one continuous session to enable maximal standardization of staining interpretation. In earlier studies, this breast cancer TMA enabled us to validate the prognostic impact of several well-established prognostic biomarkers, such as *HER2* alterations, estrogen and progesterone receptor expression (30), high Ki67LI, nuclear p53 accumulation (30), and *PTEN* deletion (34). These earlier data demonstrate the utility of our patient cohort to identify prognostic biomarkers.

The molecular database that has been collected during earlier studies for our set of cancers offers the advantage that biomarkers of interest can always be compared with preexisting data. For the purpose of this study, we had selected *HER2* amplification as well as estrogen and progesterone receptor expression because of their central role in breast cancer. The strong link between MTCO2 expression and these important features further illustrates the importance of the mitochondria quantity in breast cancer. Our analyses also included Ki67LI as another pivotal parameter for cellular activity and various further chromosomal deletions and amplifications because of the role of some of them for regulating mitochondrion homeostasis.

Mitochondrial homeostasis is critical for cancer. A sufficiently high production of mitochondria is required to suffice the needs for energy production and cell metabolism. The prominent association found between c-Myc amplification and high MTCO2 expression fits well with the key role of c-Myc as an activator of mitochondrial biogenesis in cancer (38-40). The transcription factor c-Myc is best known for its critical role in cell cycle regulation, cell growth, metabolism and apoptosis (41-43). However, c-Myc also targets more than 400 different mitochondrial genes (38-41,44). Studies have demonstrated that an elevated or reduced c-Myc protein quantity leads to an increased/diminished mitochondrial mass (45,46). This couples c-Myc's role of a key activator of cell cycle activity with mitochondrial biogenesis. As such, c-Myc increases cellular biosynthetic and respiratory capacity by upregulating mitochondrial metabolism to complement its effects on stimulating cell cycle progression to coordinate rapid cell growth (45,47).

A critical role of high mitochondrion count for cell proliferation in breast cancer is supported by our data showing a striking link between MTCO2 expression and a high Ki67LI which was also visible in the vast majority of groups defined by identical morphological or molecular features.

The PTEN-induced putative kinase 1 (PINK1)/Parkin pathway is a major inducer of mitophagy. It is triggered by mitochondrial membrane depolarization, a signal of mitochondrial dysfunction that results from lack of reducing equivalents, hypoxia and impaired electron transport [reviewed in (48)]. The conspicuous relationship between *PTEN* deletion and high MTCO2 staining in our study may thus indicate that high mitochondria quantities may also be caused by reduced mitophagy. Although clearance of damaged mitochondria via mitophagy is viewed to be also critical for cellular fitness since dysfunctional mitochondria can impair the electron transport chain function, reduced mitophagy can also promote cancer reviewed in ref. 49). Mitophagy-deficient Parkin null mice develop spontaneous hepatic tumors (50).

Decreased mitophagy may allow for a permissive threshold of dysfunctional mitochondria to persist, generating increased tumor-promoting free oxygen radicals reviewed in ref. 49).

Cytochrome oxidase subunit 2 is a key enzyme of the respiratory chain, catalyzing electron transfer from NADH and succinate to molecular oxygen (51). It has no direct tumor related function but serves as a marker for the cellular mitochondria content. Increased mitochondria content in cancer cells often occurs as a result of the elevated metabolism and energy needs of expanding tumor cell populations (52). Although the mitochondrial content provided no additional prognostic information in multivariate analysis, the marked prognostic relevance of MTCO2 immunostaining found in this study may still suggest 'mitochondria content' as a biomarker with potential clinical utility. Molecular analyses are frequently done in breast cancer to better assess patient prognosis and to determine whether adjuvant chemotherapy should be applied (6-8). Most currently used tests are analyzing RNAs of multiple genes forming a prognostic score (9-11,53). RNA based tests share the disadvantage, however, that the analyzed RNA always represents a mixture of cancer cells and a variable fraction of non-neoplastic inflammatory and stromal cells. Now that multiplex fluorescent-based quantitative IHC becomes increasingly available, it is well possible that RNA based test will sooner or later be replaced by IHC based multi-gene tests. MTCO2 might be a candidate for being part of such a test, also because of the general biologic importance of mitochondria, which are also the target of several anti-cancer drugs under development reviewed in refs. 54-57).

It is a limitation of our study that MTCO2 IHC data highlight relevant associations between cancer phenotype and genotype but do not provide mechanistic insights into the putative cancer biological role of MTCO2. Further studies on the tumor relevant aspects of mitochondrial density and MTCO2 protein function are required to better understand the prognostic role of MTCO2 in breast cancer.

In summary, our findings identify MTCO2 immunostaining as a powerful prognostic biomarker in breast cancer. MTCO2 measurement, most likely in combination with other antibodies might be of clinical utility in breast cancer prognosis assessment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed to the conception and design of the study. PL, KS, MK, IW, LW, PP, LT, CW, UH, VM, BS, IvL, TK, RHK and FJ prepared the material, and collected and analyzed the data. PL, EB, RS, MK and GS wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. RS, MK and GS confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The usage of archived diagnostic leftover tissues for manufacturing the tissue microarrays and their analysis for research purposes, as well as patient data analysis, has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics Commission of the Ärtzekammer Hamburg, Hamburg, Germany; approval no. WF-049/09). Informed consent was waived by the ethics committee due to the retrospective nature of the study. All work has been carried out in compliance with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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