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Mitochondria "fuel" breast cancer metabolism

Fifteen markers of mitochondrial biogenesis label epithelial cancer cells, but are excluded from adjacent stromal cells

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Here, we present new genetic and morphological evidence that human tumors consist of two distinct metabolic compartments. First, re-analysis of genome-wide transcriptional profiling data revealed that > 95 gene transcripts associated with mitochondrial biogenesis and/or mitochondrial translation were significantly elevated in human breast cancer cells, as compared with adjacent stromal tissue. Remarkably, nearly 40 of these upregulated gene transcripts were mitochondrial ribosomal proteins (MRPs), functionally associated with mitochondrial translation of protein components of the OXPHOS complex. Second, during validation by immunohistochemistry, we observed that antibodies directed against 15 markers of mitochondrial biogenesis and/or mitochondrial translation (AKAP1, GOLPH3, GOLPH3L, MCT1, MRPL40, MRPS7, MRPS15, MRPS22, NRF1, NRF2, PGC1-α, POLRMT, TFAM, TIMM9 and TOMM70A) selectively labeled epithelial breast cancer cells. These same mitochondrial markers were largely absent or excluded from adjacent tumor stromal cells. Finally, markers of mitochondrial lipid synthesis (GOLPH3) and mitochondrial translation (POLRMT) were associated with poor clinical outcome in human breast cancer patients. Thus, we conclude that human breast cancers contain two distinct metabolic compartments—a glycolytic tumor stroma, which surrounds oxidative epithelial cancer cells—that are mitochondria-rich. The co-existence of these two compartments is indicative of metabolic symbiosis between epithelial cancer cells and their surrounding stroma. As such, epithelial breast cancer cells should be viewed as predatory metabolic "parasites," which undergo anabolic reprogramming to amplify their mitochondrial "power." This notion is consistent with the observation that the anti-malarial agent chloroquine may be an effective anticancer agent. New anticancer therapies should be developed to target mitochondrial biogenesis and/or mitochondrial translation in human cancer cells.

Introduction

We and other investigators have recently proposed that mitochondria are both the "powerhouse" and "Achilles' heel" of human cancer cells.[1-3]

More specifically, cancer cells amplify their capacity for mitochondrial oxidative metabolism (OXPHOS) and "steal" high-energy mitochondrial fuels from adjacent stromal cells, which are undergoing aerobic glycolysis (the "reverse Warburg effect").[4,5] We have termed this new model of cancer metabolism "two-compartment tumor metabolism," to reflect that two distinct opposing metabolic compartments co-exist, side-by-side, within human tumors.[6-11]

In direct support of this hypothesis, genetic induction of mitochondrial dysfunction in cancer-associated fibroblasts dramatically promotes both local tumor growth and distant cancer cell metastasis.[12-24] Conversely, genetic amplification of mitochondrial biogenesis in epithelial cancer cells also promotes tumor growth, independently of neo-angiogenesis.[23,25-28]

Consistent with these pre-clinical findings, we have identified a series of new stromal biomarkers and related gene signatures that are characteristic of this type of lethal cancer metabolism.[29-34] Remarkably, these diagnostics effectively predict early

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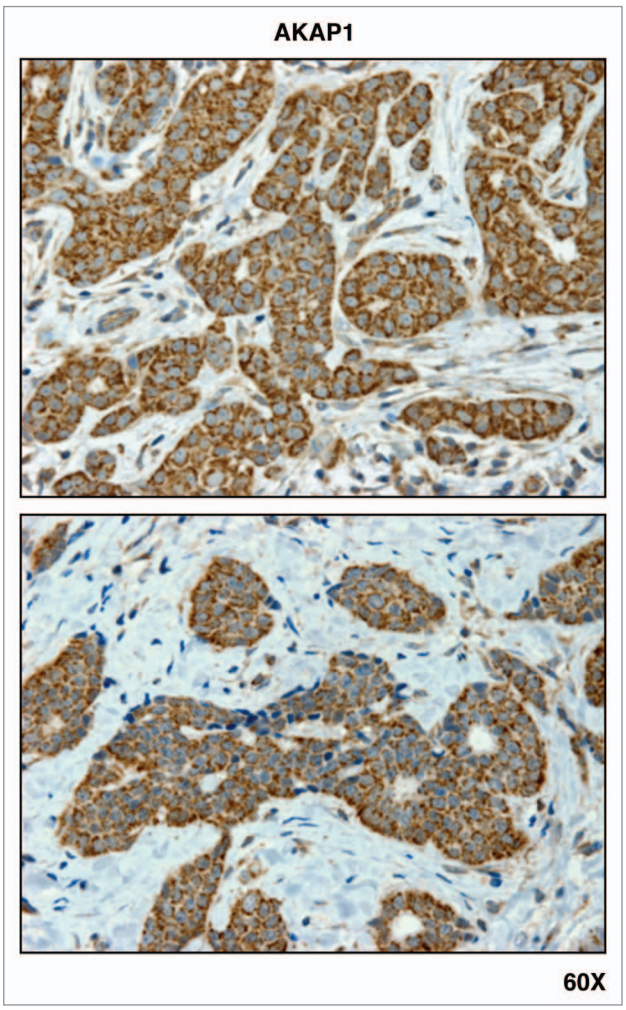

Figure 1. AKAP1, a mitochondrial marker, is predominantly confined to epithelial cancer cells, and largely absent from adjacent stromal cells, in human breast cancer tissues. Paraffin-embedded sections of human breast cancer tumor tissue were immunostained with antibodies directed against AKAP1. Slides were then counter-stained with hematoxylin. Note that AKAP1 is highly expressed in the epithelial compartment (brown color). Two representative images are shown. Original magnification is 60x, as indicated.

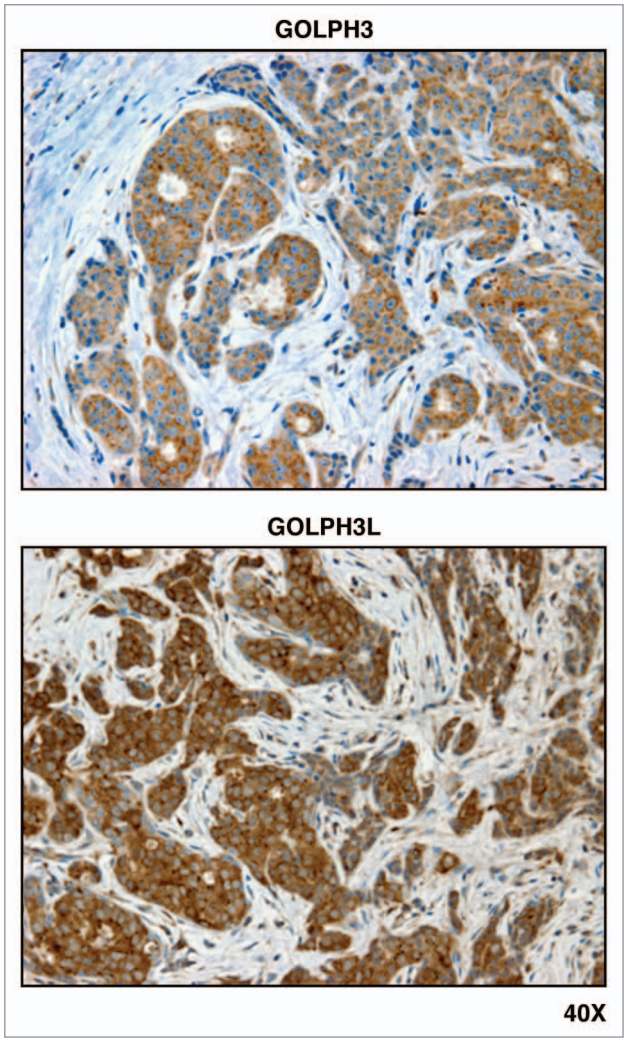

Figure 2. GOLPH3 and GOLPH3L, markers of mitochondrial lipid biosynthesis, are localized mainly to epithelial cancer cells in human breast cancer tissues. Paraffin-embedded sections of human breast cancer tumor tissue were immunostained with antibodies directed against GOLPH3 and GOLPH3L. Slides were then counter-stained with hematoxylin. Note that both GOLPH3 family members are largely absent from the stromal compartment and confined to the epithelial compartment (brown color). Original magnification is 40x, as indicated.

tumor recurrence, lymph node metastasis, tamoxifen resistance and overall poor clinical outcome in human breast cancer patients.[8,10] In this regard, the prognostic value of a loss of stromal caveolin-1 (Cav-1; indicative of glycolysis and autophagy in the tumor microenvironment) has now been independently validated in seven different countries, and its predictive capacity has also been extended to DCIS progression, human prostate cancers and metastatic melanoma.[8,10,35-41] In addition, the expression of stromal MCT4 appears to inversely correlate with stromal Cav-1, allowing them to be used together as companion diagnostics for the detection of "two-compartment tumor metabolism."[31]

However, in addition to these stromal diagnostics, new epithelial biomarkers are desperately needed to identify the corresponding onset of mitochondrial biogenesis in human breast cancer cells.

Here, we show that 15 markers of mitochondrial biogenesis selectively label epithelial breast cancer cells and are largely absent from adjacent tumor stromal cells. Future studies will be necessary to determine if these promising new epithelial biomarkers can also be used to predict clinical outcome.

Results

Transcriptional profiling reveals that mitochondrial biogenesis and mitochondrial translation are amplified in epithelial breast cancer cells. To investigate the potential role of epithelial mitochondrial biogenesis in the pathogenesis of human breast cancers, we re-analyzed the transcriptional profiles of epithelial cancer cells and adjacent stromal cells that were physically

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Table 1. Transcripts of proteins involved in mitochondrial biogenesis and mitochondrial protein translation are upregulated in human breast cancer cells as compared with adjacent stromal cells

Gene	Fold change	P value	Gene description
AKAP1	3.334518	7.75E-04	A kinase (PRKA) anchor protein 1
FARS2	2.057832	2.22E-02	phenylalanyl-tRNA synthetase 2, mitochondrial
GOLPH3L	3.977418	1.04E-04	golgi phosphoprotein 3-like
GOLPH3	2.944535	2.38E-03	golgi phosphoprotein 3 (coat-protein)
GRPEL1	2.392038	1.01E-02	GrpE-like 1, mitochondrial (E. coli)
IMMT	4.709106	8.89E-06	inner membrane protein, mitochondrial (mitofilin)
ISCA1	2.865072	2.96E-03	iron-sulfur cluster assembly 1 homolog (S. cerevisiae)
IARS2	4.700889	9.15E-06	isoleucyl-tRNA synthetase 2, mitochondrial
LARS2	2.09848	2.03E-02	leucyl-tRNA synthetase 2, mitochondrial
MRPS33	5.599499	3.71E-07	mitochondrial ribosomal protein S33
MRPL49	4.94263	3.93E-06	mitochondrial ribosomal protein L49
MRPS15	4.397211	2.59E-05	mitochondrial ribosomal protein S15
MRP63	4.298648	3.62E-05	mitochondrial ribosomal protein 63
MRPL33	4.152023	5.89E-05	mitochondrial ribosomal protein L33
MRPS26	3.968262	1.08E-04	mitochondrial ribosomal protein S26
MRPL20	3.926431	1.23E-04	mitochondrial ribosomal protein L20
MRPL18	3.849704	1.58E-04	mitochondrial ribosomal protein L18
MRPS14	3.663705	2.84E-04	mitochondrial ribosomal protein S14
MRPS18B	3.652361	2.94E-04	mitochondrial ribosomal protein S18B
MRPL3	3.608762	3.37E-04	mitochondrial ribosomal protein L3
MRPL54	3.60572	3.40E-04	mitochondrial ribosomal protein L54
MRPL48	3.548492	4.06E-04	mitochondrial ribosomal protein L48
MRPS30	3.526142	4.34E-04	mitochondrial ribosomal protein S30
MRPL46	3.432304	5.78E-04	mitochondrial ribosomal protein L46
MRPL39	3.416735	6.06E-04	mitochondrial ribosomal protein L39
MRP63	3.348354	7.43E-04	mitochondrial ribosomal protein 63
MRPS7	3.31261	8.27E-04	mitochondrial ribosomal protein S7
MRPS27	3.312577	8.27E-04	mitochondrial ribosomal protein S27
MRPS22	3.272431	9.31E-04	mitochondrial ribosomal protein S22
MRPS31	3.187071	1.20E-03	mitochondrial ribosomal protein S31
MRPL24	3.177055	1.23E-03	mitochondrial ribosomal protein L24
MRPL40	3.107139	1.51E-03	mitochondrial ribosomal protein L40
MRPL22	3.047896	1.78E-03	mitochondrial ribosomal protein L22
MRPL9	3.026088	1.89E-03	mitochondrial ribosomal protein L9
MRPL17	2.944333	2.38E-03	mitochondrial ribosomal protein L17
MRPS12	2.867822	2.94E-03	mitochondrial ribosomal protein S12
MRPS11	2.782746	3.71E-03	mitochondrial ribosomal protein S11
MRPS35	2.77418	3.79E-03	mitochondrial ribosomal protein S35
MRPL13	2.686639	4.79E-03	mitochondrial ribosomal protein L13
MRPL52	2.518922	7.38E-03	mitochondrial ribosomal protein L52
MRPL16	2.484585	8.05E-03	mitochondrial ribosomal protein L16

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Table 1. Transcripts of proteins involved in mitochondrial biogenesis and mitochondrial protein translation are upregulated in human breast cancer cells as compared with adjacent stromal cells

Gene	Fold change	P value	Gene description
MRPL9	2.462051	8.52E-03	mitochondrial ribosomal protein L9
MRPL15	2.259912	1.39E-02	mitochondrial ribosomal protein L15
MRPS28	2.24818	1.43E-02	mitochondrial ribosomal protein S28
MRPS14	2.140701	1.84E-02	mitochondrial ribosomal protein S14
MRPS17	2.032222	2.35E-02	mitochondrial ribosomal protein S17
MRPS18A	1.979926	2.64E-02	mitochondrial ribosomal protein S18A
MRPL42	1.978797	2.65E-02	mitochondrial ribosomal protein L42
MPV17	3.267626	9.44E-04	MpV17 mitochondrial inner membrane protein
MTCH2	4.257272	4.15E-05	mitochondrial carrier homolog 2 (C. elegans)
MTCH1	3.010569	1.98E-03	mitochondrial carrier homolog 1 (C. elegans)
MTO1	2.005924	2.49E-02	mitochondrial translation optimization 1
NFU1	4.623228	1.20E-05	NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae)
NRF1	2.493935	7.86E-03	nuclear respiratory factor 1
PDF	2.556598	6.71E-03	peptide deformylase (mitochondrial)
PMPCB	3.81323	1.77E-04	peptidase (mitochondrial processing) beta
POLRMT	3.253681	9.84E-04	polymerase (RNA) mitochondrial (DNA directed)
SLC25A3	3.760733	2.09E-04	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3
SLC25A6	3.749114	2.17E-04	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
SLC25A5	3.492682	4.81E-04	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
SLC25A14	2.87376	2.89E-03	solute carrier family 25 (mitochondrial carrier, brain), member 14
SLC25A17	2.724374	4.33E-03	solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34kDa), member 17
SLC25A11	2.703927	4.57E-03	solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
SLC25A15	2.158981	1.77E-02	solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15
TFAM	2.220086	1.53E-02	transcription factor A, mitochondrial
TFB1M	1.974612	2.67E-02	transcription factor B1, mitochondrial
TIMM17A	4.946017	3.88E-06	translocase of inner mitochondrial membrane 17A
TIMM9	3.578793	3.69E-04	translocase of inner mitochondrial membrane 9
TIMM23	3.013111	1.97E-03	translocase of inner mitochondrial membrane 23
TIMM13	2.782496	3.71E-03	translocase of inner mitochondrial membrane 13
TIMM22	2.648125	5.29E-03	translocase of inner mitochondrial membrane 22
TIMM8B	2.227674	1.50E-02	translocase of inner mitochondrial membrane 8B
TOMM20	5.068116	2.52E-06	translocase of outer mitochondrial membrane 20
TOMM70A	3.056903	1.74E-03	translocase of outer mitochondrial membrane 70
TOMM7	3.034435	1.85E-03	translocase of outer mitochondrial membrane 7
TUFM	3.38096	6.74E-04	Tu translation elongation factor, mitochondrial
UCRC	3.353218	7.33E-04	ubiquinol-cytochrome c reductase complex (7.2 kD)

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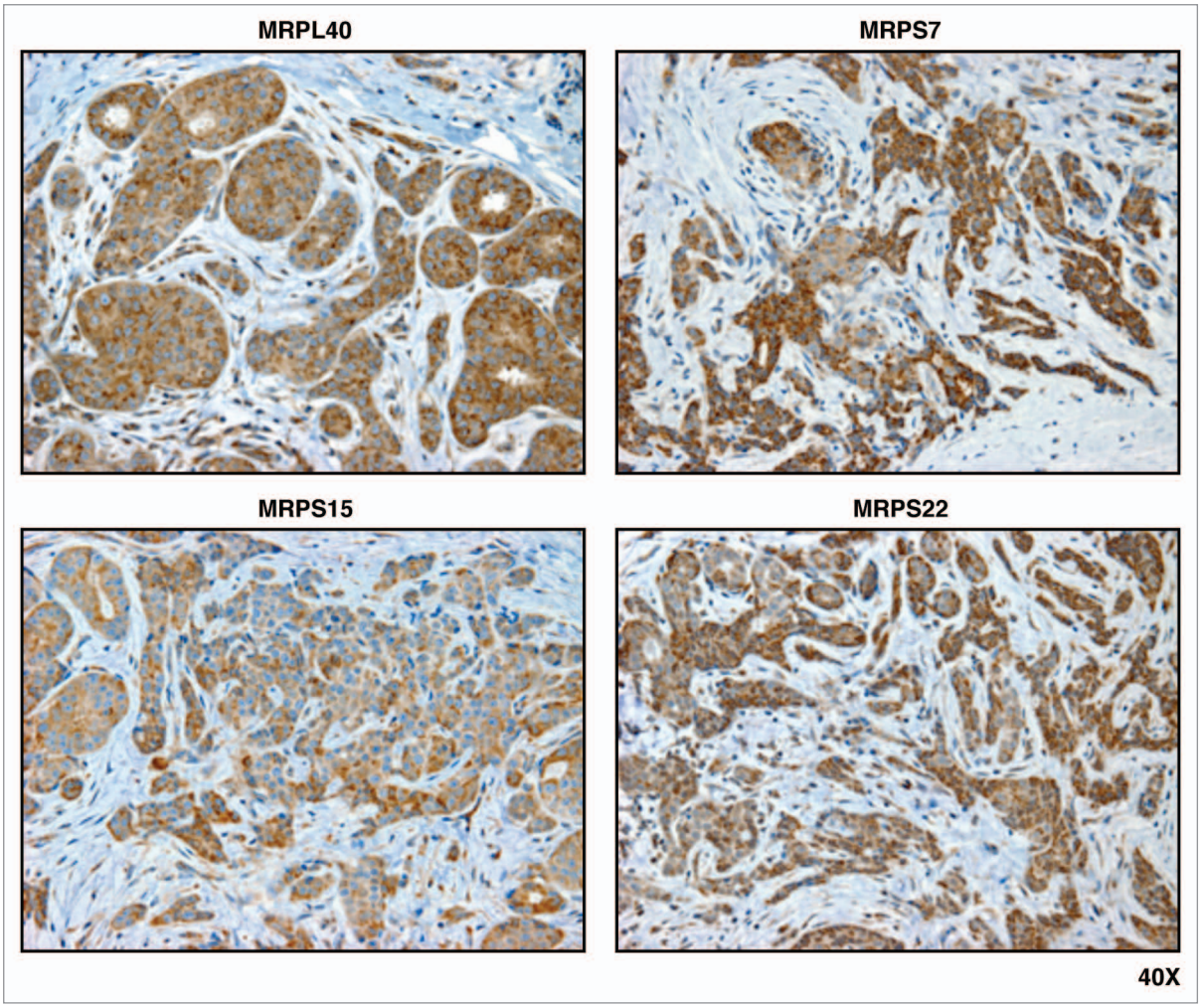

Figure 3. Mitochondrial ribosomal proteins (MRPL40, MRPS7, MRPS15, and MRPS22) are localized to epithelial cancer cells, but absent from adjacent tumor stroma, in human breast cancers. Paraffin-embedded sections of human breast cancer primary tumors were immunostained with antibodies directed against MRPL40, MRPS7, MRPS15, and MRPS22 (all mitochondrial ribosomal proteins). Note that immunostaining (brown color) is largely confined to the epithelial cancer cells. Original magnification, 40x.

separated by laser capture microdissection (from n = 28 human breast cancer patients).[42]

As shown in **Table 1**, important functional components involved in both mitochondrial biogenesis and/or mitochondrial translation were all transcriptionally upregulated in human breast cancer epithelial cells and, hence, downregulated in adjacent stromal cells. Only gene transcripts upregulated by > 1.5-fold are shown.

Most notably, transcripts encoding 39 mitochondrial ribosomal proteins (MRPs), all involved in mitochondrial translation of OXPHOS complex components, were specifically upregulated in epithelial cancer cells, between 2–5-fold (**Table 1**). Similarly, a series of transcription factors that are known to be associated with mitochondrial biogenesis were elevated, including NRF1, TFAM and TFB1M as well as TIMM and TOMM family members. In addition, gene transcripts associated with mitochondrial lipid biosynthesis (GOLPH3 and GOLPH3L) were also increased by ~3–4-fold in epithelial breast cancer cells.

Other mitochondrial-related genes involved in oxidative energy metabolism, such as components of the mitochondrial ATP synthase (ATP5) and ketone body re-utilization (OXCT1, ACAT2, MCT1/5), we also upregulated in human breast cancer cells, relative to stromal cells (**Tables 2 and 3**). This is consistent with our previous findings regarding the upregulation of OXPHOS components (complexes I-IV) in human breast cancer cells.[6]

In total, > 95 gene transcripts associated with mitochondrial biogenesis and/or translation were found to be upregulated specifically in the epithelial cancer cell compartment.

Markers of mitochondrial biogenesis and mitochondrial translation are selectively localized to epithelial cancer cells and are absent from adjacent stromal tissue. To validate the gene profiling results presented above, we next performed immunohistochemical staining, using specific antibody probes, on a series of human breast cancer patient samples that were selected, because they lack the expression of Cav-1 in the tumor stroma (an established marker of stromal autophagy and two-compartment tumor metabolism).

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Table 2. Transcripts encoding the mitochondrial ATP synthase are upregulated in human breast cancer cells, as compared with adjacent stromal cells

Symbol	Fold change	P value	Gene description
ATP5F1	5.39378	7.83E-07	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit B1
ATP5O	5.115639	2.13E-06	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity)
ATP5B	5.0431	2.75E-06	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
ATP5A1	5.010375	3.09E-06	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
ATP5C1	4.638387	1.14E-05	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
ATP5L	4.618911	1.22E-05	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G
ATP5J	4.505825	1.79E-05	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
ATP5H	4.007293	9.48E-05	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d
ATP5C1	3.953547	1.13E-04	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
ATP5G3	3.51811	4.45E-04	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C3 (subunit 9)
ATP5J2	3.347841	7.45E-04	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F2
ATP5G1	3.0078	2.00E-03	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C1 (subunit 9)
ATP5I	2.735813	4.20E-03	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit E
ATP5D	2.633354	5.50E-03	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit
ATP5G2	2.46535	8.45E-03	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C2 (subunit 9)
ATP5L	2.394331	1.01E-02	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G
ATP5E	2.294952	1.28E-02	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit

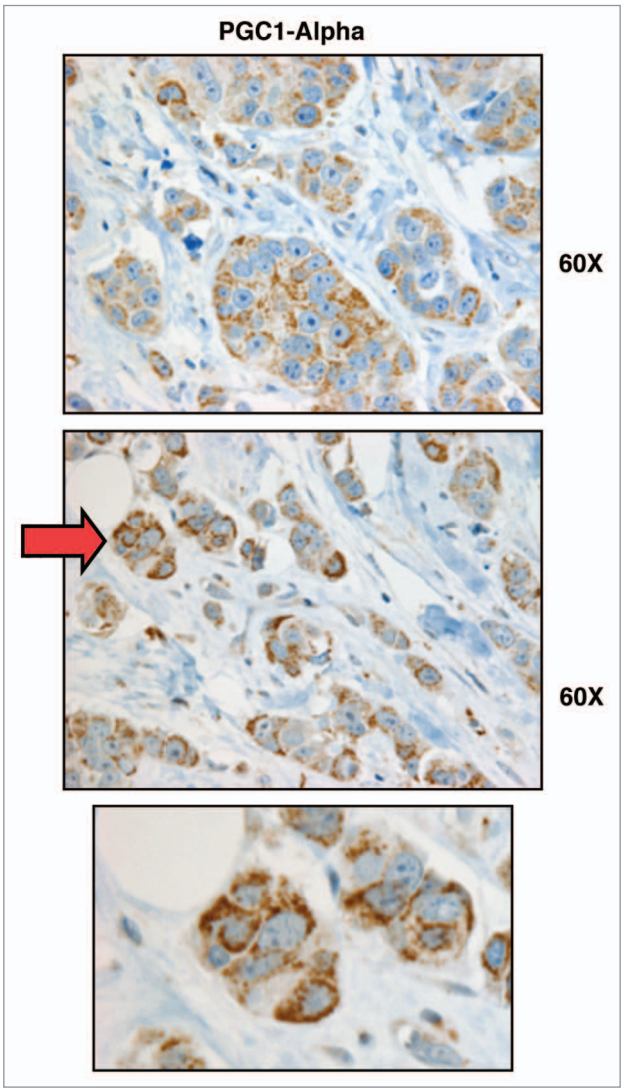

Figure 4. PGC1-α, a key mitochondrial transcription factor, is largely confined to epithelial cancer cells, and absent from stromal cells, in human breast cancers. Paraffin-embedded sections of human breast cancer primary tumors were immunostained with antibodies directed against PGC1-α. Note that PGC1-α immunostaining is largely confined to the epithelial cancer cells. A red arrow points at an area that is further magnified below and is shown as an inset. Original magnification, 60x.

Results of this detailed analysis are shown in **Figures 1–7**. Importantly, we validated that 15 markers of mitochondrial biogenesis and/or mitochondrial translation were specifically overexpressed in human epithelial breast cancer cells. These markers included protein products involved in mitochondrial signaling (AKAP1; **Fig. 1**), mitochondrial lipid synthesis (GOLPH3 and GOLPH3L; **Fig. 2**) and mitochondrial translation (MRPL40, MRPS7, MRPS15 and MRPS22; **Fig. 3**).

Immunostaining results are also presented for mitochondrial transcription factors (PGC1-α, NRF1/2, and TFAM; **Figs. 4–6**), as well as POLRMT (the mitochondrial RNA-polymerase) and TOMM and TIMM family members (**Fig. 6**). The distribution of MCT1 is shown for comparison (**Fig. 7**). MCT1 (monocarboxylate transporter 1) allows for the uptake of high-energy mitochondrial fuels, such as L-lactate and ketone bodies, to "feed" oxidative mitochondrial metabolism in epithelial cancer cells. Again, MCT1 is largely confined to the epithelial cancer cell compartment.

Markers of mitochondrial lipid synthesis (GOLPH3) and mitochondrial translation (POLRMT) predict poor clinical outcome in human breast cancer patients. Finally, we also performed survival analysis, using existing transcriptional profiling data and accessible outcome data from human breast cancer patients. **Figure 8** shows that that when gene transcripts associated with mitochondrial lipid synthesis (GOLPH3) and

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Table 3. Transcripts encoding proteins associated with ketone body re-utilization are upregulated in human breast cancer cells, as compared with adjacent stromal cells

Symbol	Fold change	P value	Gene description
ACAT2	3.281986	9.05E-04	acetyl-Coenzyme A acetyltransferase 2
OXCT1	2.087893	2.08E-02	3-oxoacid CoA transferase 1
SLC16A4	2.052101	2.25E-02	solute carrier family 16, member 4 (monocarboxylic acid transporter 5; MCT5)
SLC16A1	1.628443	5.46E-02	solute carrier family 16, member 1 (monocarboxylic acid transporter 1; MCT1)

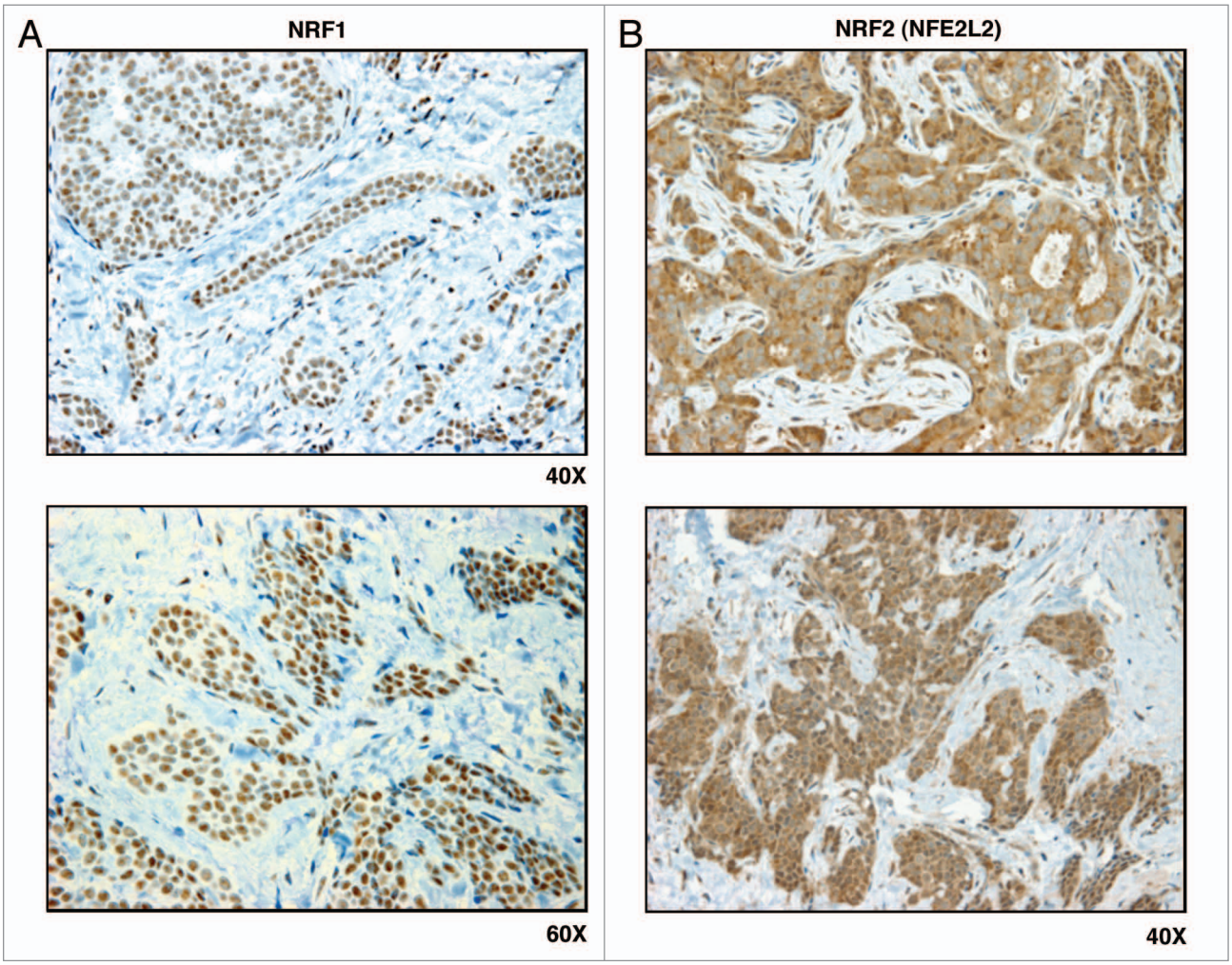

Figure 5. NRF1 and NRF2 family members preferentially label epithelial cancer cells in human breast cancers, but not adjacent stromal cells. Paraffin-embedded sections of human breast cancer primary tumors were immunostained with antibodies directed against either NRF1 (**A**) or NRF2 (**B**). Note that NRF1/2 immunostaining is largely confined to the epithelial cancer cells. Original magnification is as indicated.

mitochondrial protein translation (POLRMT) are transcriptionally upregulated in human breast cancers, there is a specific association with poor overall survival, especially in ER+/Luminal A breast cancer patients. The number of cases with annotation is shown.

Discussion

Here, we present both genetic and morphological evidence that mitochondrial biogenesis and/or mitochondrial translation are amplified in epithelial breast cancer cells, but not in adjacent stromal tissue (**Fig. 9**). Via re-analysis of existing laser-capture microdissection data, we see that > 95 transcripts associated with mitochondrial biogenesis and mitochondrial translation are elevated specifically in the breast cancer cell compartment, and hence downregulated in adjacent cancer-associated fibroblasts. This transcriptional profiling data was then validated by immunohistochemical analysis of human breast cancer samples. We now show that 15 marker proteins associated with mitochondrial biogenesis are highly expressed in human breast cancer cells and are largely absent in adjacent stromal tissue. These findings are consistent with "two-compartment tumor metabolism," which

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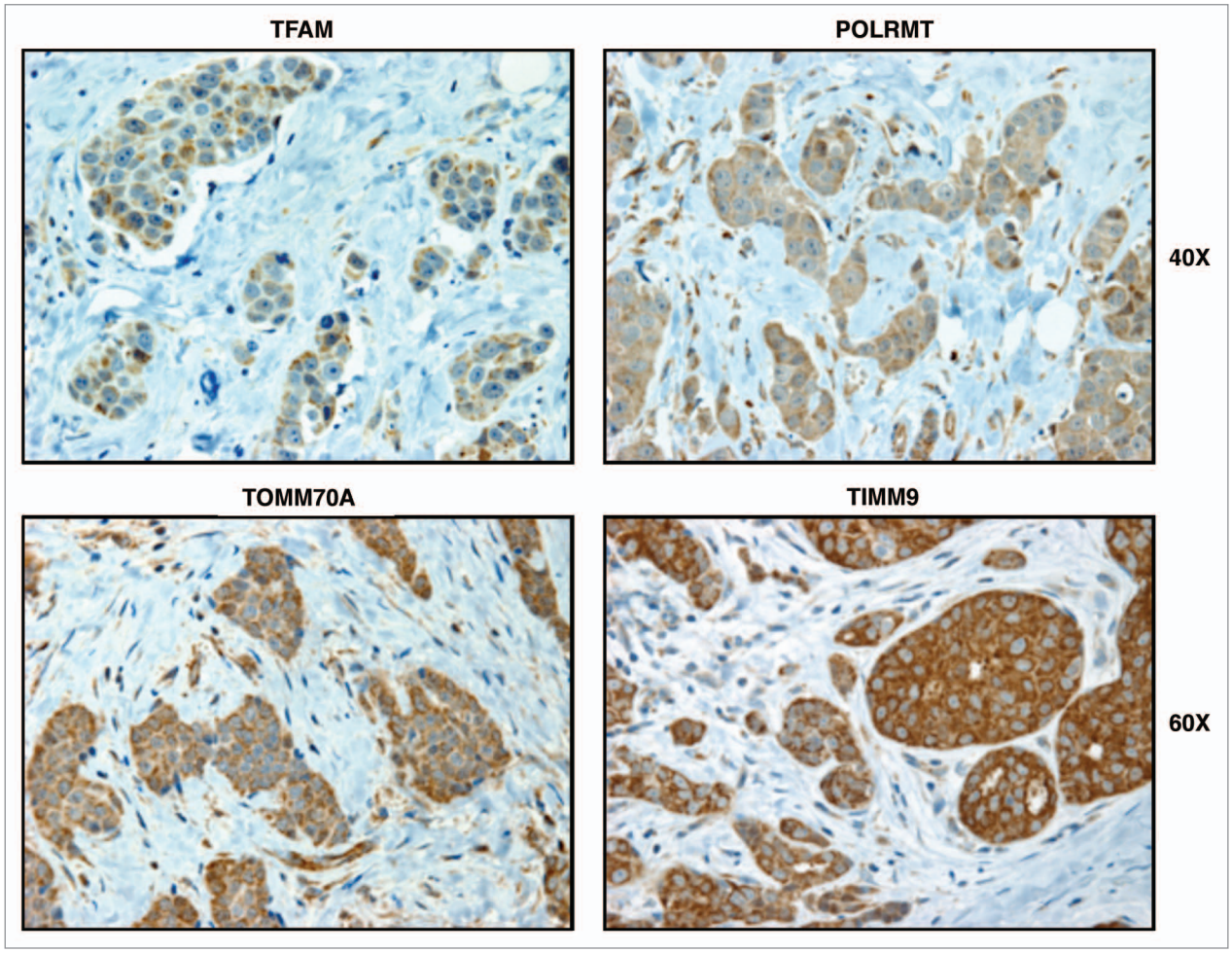

Figure 6. Markers of mitochondrial biogenesis (TFAM, POLRMT, TOMM70A, and TIMM9) are all predominantly confined to epithelial cancer cells in human breast cancer tumor tissues, but are largely absent from adjacent stromal cells. Paraffin-embedded sections of human breast cancer tumor tissue were immunostained with antibodies directed against TFAM, POLRMT, TOMM70A and TIMM9. Slides were then counter-stained with hematoxylin. Note that TFAM, POLRMT, TOMM70A and TIMM9 are all largely absent from the stromal compartment and confined to the epithelial compartment (brown color). Original magnifications, 40x and 60x, are as indicated.

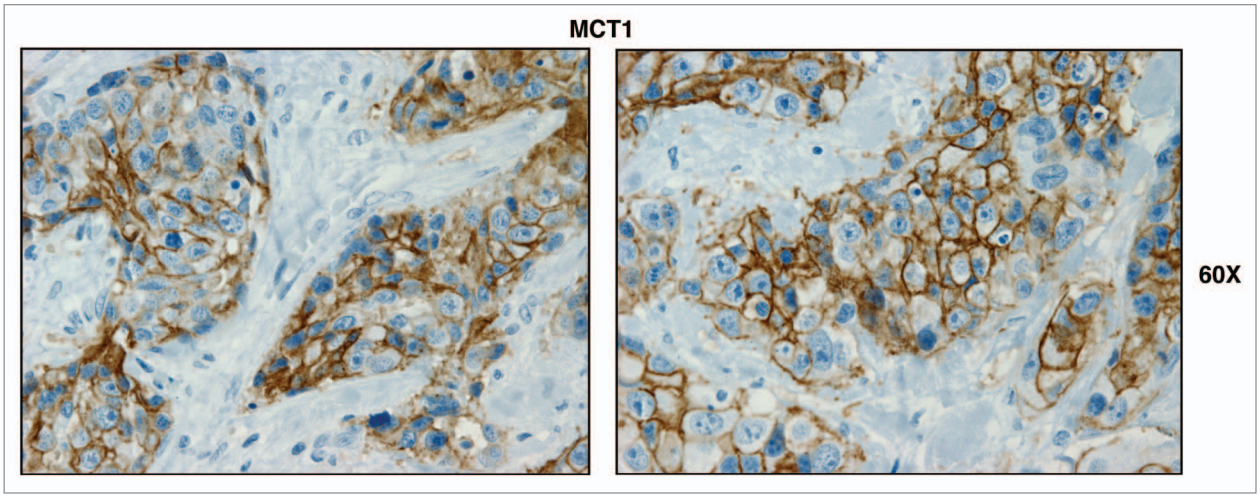

Figure 7. MCT1, a metabolic marker for the uptake of high-energy mitochondrial fuels, is predominantly localized to epithelial cancer cells, and absent from adjacent tumor stromal cells, in human breast cancers. Paraffin-embedded sections of human breast cancer tumor tissue were immunostained with antibodies directed against MCT1. Slides were then counter-stained with hematoxylin. Note that MCT1 immunostaining is largely absent from the stromal compartment and confined to the epithelial compartment (brown color). Original magnification is 60x, as indicated.

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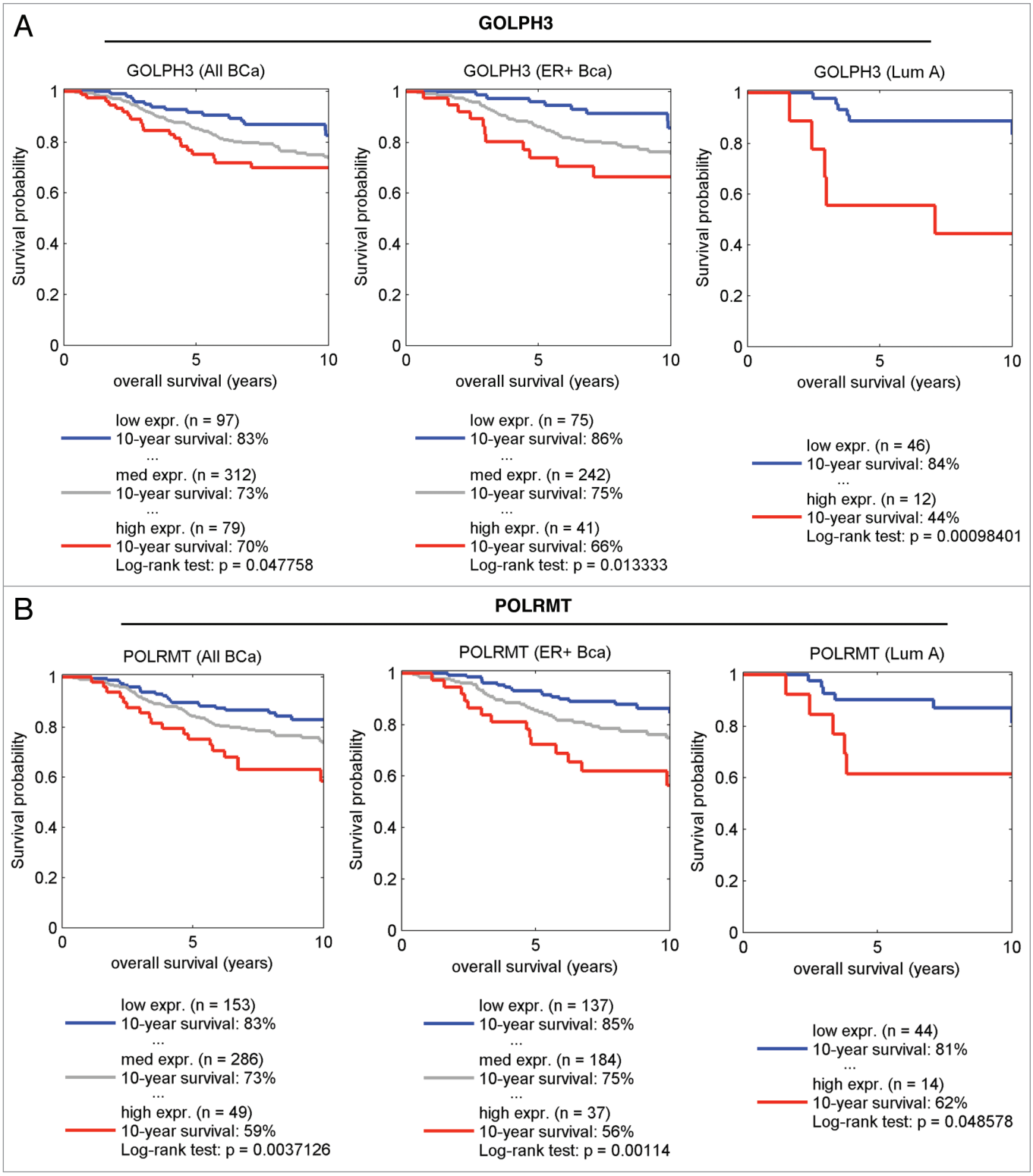

Figure 8. GOLPH3 (a marker of mitochondrial lipid synthesis) and POLRMT (a marker of mitochondrial translation) both predict poor clinical outcome in human breast cancer patients. Note that the expression levels of the gene transcripts for GOLPH3 (**A**) and POLRMT (**B**) predict poor overall survival, especially in ER-positive (**A**) patients. Numbers of cases with annotation are shown. P values are as indicated. X-Tile software was employed to identify subpopulation cut-points to observe maximum survival differences between the high expression and low expression subpopulations. The Log-rank test was used to evaluate the significance of differences in survival curves for high vs. low expressing populations.

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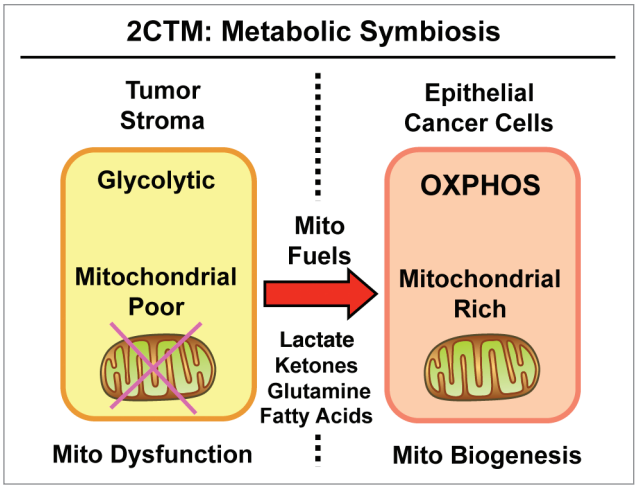

Figure 9. Two-compartment tumor metabolism (2CTM) reflects metabolic symbiosis. We suggest that aggressive breast cancers consist of two distinct metabolic compartments. In the tumor microenvironment, stromal fibroblasts (and other cell types) show signs of mitochondrial dysfunction, are mitochondrial-deficient, and metabolically shift toward aerobic glycolysis (the "reverse Warburg effect"). This results in the stromal production of high-energy mitochondrial fuels, such as L-lactate, ketone bodies, glutamine and free fatty acids. These recycled nutrients are then available to "feed" neighboring cancer cells. In response to this energy-rich microenvironment, epithelial cancer cells undergo mitochondrial biogenesis, amplifying their capacity for oxidative mitochondrial metabolism (OXPHOS). Thus, the tumor stroma and epithelial breast cancer cells are metabolically linked in a "symbiotic/parasitic" relationship, related to energy transfer or an energy imbalance.

postulates that epithelial cancer cells amplify oxidative mitochondrial metabolism (OXPHOS), while cancer-associated fibroblasts are predominantly glycolytic, and "suffer" from a mitochondrial deficiency or mitochondrial dysfunction.[1,2,8]

In accordance with the idea that markers of mitochondrial biogenesis may have predictive value as diagnostics for breast cancers and other types of human cancer, several other groups in the United Kingdom (UK) have recently shown that the mitochondrial markers TIMM17A and TOMM34 are associated with poor clinical outcome and may be predictive of higher tumor grade and size, lympho-vascular invasion as well as lymph node metastasis.[43-45] Consistent with these findings, we have previously shown that patients whose breast cancer cells are using high-energy fuels (such as L-lactate and ketone bodies) are more prone to early tumor recurrence, metastasis and poor clinical outcome.[26,27,32]

Similarly, Schimmer and colleagues have recently screened a chemical library of FDA-approved drugs and identified the antimicrobial tigecycline as a novel agent that selectively kills cancer cells but not normal cells. Mechanistically, tigecycline conferred lethality, as it functions as a selective inhibitor of mitochondrial translation, thereby inhibiting mitochondrial biogenesis.[46-48]

These results are consistent with our recent proposal that aggressive epithelial cancer cells behave much like infectious metabolic "parasites," and that we should identify novel anticancer agents (akin to antibiotics) to eradicate mitochondrial biogenesis in epithelial cancer cells.[1] This could explain the retrospective success of Metformin, which prevents the onset of nearly all types of cancers in diabetic patients, likely because it functions as a "weak" mitochondrial poison (an inhibitor of complex I) (reviewed in ref. 1).

Many of the markers of mitochondrial biogenesis that we have used here may ultimately prove to be new valuable biomarkers that can predict clinical outcome in human breast cancer patients. For example, GOLPH3, which is a marker of increased mitochondrial lipid synthesis, has already been shown to predict poor clinical outcome in oral, esophageal and prostate cancers as well as glioblastomas.[49-52] Similarly, we have recently demonstrated that overexpression of GOLPH3 in the triple-negative breast cancer cell line, MDA-MB-231 cells, increases mitochondrial function and promotes tumor growth, without a significant increase in tumor angiogenesis.[23]

Materials and Methods

Materials. The following rabbit polyclonal antibodies we used were generated by the Human Protein Altas (http://www.proteinatlas.org) and were obtained commercially from Sigma-Aldrich: AKAP1 (HPA008691), GOLPH3 (HPA044564), GOLPH3L (HPA028558), MRPL40 (HPA006181), MRPS7 (HPA022522), MRPS15 (HPA028134), MRPS22 (HPA006083), NRF1 (HPA029329), NRF2 (HPA002990), POLRMT (HPA006366), TFAM (HPA040648), TIMM9 (HPA002932) and TOMM70A (HPA014589). Antibodies to PGC1-α were from Santa Cruz Biotech (sc-13067). Finally, mono-specific rabbit polyclonal antibodies to MCT1 were the generous gift of Dr. Nancy Philip (Thomas Jefferson University).

Immunohistochemistry. Paraffin-embedded sections were immunostained as previously described. Briefly, sections were de-paraffinized, rehydrated and washed in PBS. Antigen retrieval was performed in 10 mM sodium citrate, pH 6.0 for 10 min using a pressure cooker. After blocking with 3% hydrogen peroxide for 10 min, sections were incubated with 10% goat serum for 1 h. Then, sections were incubated with primary antibodies overnight at 4°C. Antibody binding was detected using a biotinylated secondary (Vector Labs) followed by strepavidin-HRP (Dako). Immunoreactivity was revealed using 3, 3' diaminobenzidine.

Kaplan-Meier analysis. Kaplan-Meier analysis was performed, essentially as we previously described.[28] Briefly, X-Tile software was employed to identify subpopulation cut-points to observe maximum survival differences between the high expression and low expression subpopulations. The Log-rank test was used to evaluate the significance of differences in survival curves for high vs. low expressing populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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