The Role of CPT1A as a Biomarker of Breast Cancer Progression – A Bioinformatic Analysis

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Abstract

Background

Breast cancer is the commonest malignancy of women and with its incidence on the rise, the need to identify new targets for treatment is imperative. There is a growing interest in the role of lipid metabolism in cancer. Carnitine palmitoyl-transferase-1 (CPT-1); the rate limiting step in fatty acid oxidation, has been shown to be overexpressed in a range of tumours. There are 3 isoforms of CPT-1; A, B and C. It is CPT-1A that has been shown to be the predominant isoform which is overexpressed in breast cancer.

Methods

We performed a bioinformatic analysis using readily available online platforms to establish the prognostic and predictive effects related to CPT-1A expression. These include the KM plotter tool, the Human Protein Atlas, the cBioPortal tool, the G2O tools, the MethSurv tool, the ROC plotter tool. A Network analysis was performed using the Oncomine platform and the gene concepts for the cancer hallmarks, including immune regulation, were based on the NanoString concepts. The gene concepts for the epigenetic pathways were obtained from the EpiFactor website. Spearman correlations (r) to determine the relationship between CPT-1A and the immune response were obtained using the TISIDB portal.

Results

Overexpression of CPT-1A largely confers a worse prognosis and that CPT-1A progressively recruits a range of pathways as breast cancer progresses. The scope of this gene's interactions with cancer pathways is far wider than previously realised and includes associations with epigenetic regulation and immune evasion pathways, as well as wild-type moderate to high penetrant genes involved in hereditary breast cancer. CPT-1A genomic alterations are detected in 9% of breast carcinomas and both the alteration and the metagene associated with it, confers a poor prognosis.

Conclusions

CPT-1A expression can be utilised as a biomarker of disease progression and as a potential therapeutic target.

Background

Breast cancer is the commonest malignancy in women and its incidence continues to rise with over 1 million new cases each year(1). In 2020 the world health organisation (WHO) stated that the most commonly diagnosed cancer was breast with 2.26 million new cases(2). Breast cancer can be classified by its histological subtype, its receptor status or its molecular phenotype(3). The mainstay of oncological management of breast cancer includes endocrine therapy, human epidermal growth factor receptor 2 (HER2) targeted therapy and chemotherapy. However, there is a need to identify further targets, particularly targets which can deal with the problem of tumour evolution and disease progression and that can be potentially used as therapeutic options.

It is well-established that global alterations in energy metabolism by cancer cells can promote tumourigenesis(4). There is intense interest in probing metabolic pathways for biomarkers and therapeutic targets. While many metabolic pathways have received a lot of attention, lipid metabolism has been relatively under investigated but contains some promising targets(5). Indeed, differences in expression of lipid metabolism related proteins in breast cancer subtypes has been demonstrated(6). As the enzymes of oxidative metabolism are younger evolutionarily, the pathways have less redundancy and may be promising targets to control tumour evolution.

The carnitine shuttle system represents the rate limiting step in fatty acid oxidation (FAO). A key component in this system is CPT-1 which resides on the outer mitochondrial membrane. It facilitates the formation of an acyl-carnitine complex which is then able to traverse the inner mitochondrial membrane via other members of the shuttle system and then eventually undergo beta oxidation(7).

Three different isoforms of CPT-1 have been identified. CPT-1A appears to have the widest distribution in the body. It was originally considered as the hepatic isoform as its deficiency resulted in a rare genetic disorder of infants leading to hepatomegaly, seizures and sudden death. It has been found in the liver, pancreas, brain, kidney, blood, embryonic tissues, colon, duodenum and small intestine(8). CPT-1B originally identified as the muscle isoform(9) is expressed only in brown adipose tissue, muscle and heart and CPT-1C, known as the brain isoform(10), is expressed in the brain and has also shown expression in testes. Overexpression of CPT-1A has been linked to progression of malignancy such as breast, prostate and lymphoma. It has also been shown that suppression of CPT-1 can lead to cell death and suppression of hallmarks of cancer progression(11). Furthermore an integrated genomics approach has identified CPT-1A as a driver of proliferation in luminal breast cancers(12) and it has also recently been suggested that CPT-1A could be used as a biomarker for disease monitoring in breast cancer(13).

Drug therapies targeting CPT-1 have been developed for the treatment of heart disease and diabetes(14, 15). There has been increasing interest in targeting FAO in the context of cancer. The CPT-1 inhibitor etomoxir (ETO), which targets both CPT-1A and B, has been used in this context and has shown to promote oxidative stress and impair cell proliferation in T cells(16). ETO has also been shown to decrease proliferation without reducing cell viability in

triple negative breast cancer cell lines that over express the oncogenic transcription factor MYC(16, 17). ST12326 also known as Teglicar; a newer, selective and reversible inhibitor of CPT-1A, has been shown to inhibit fatty acid oxidation in leukaemia cells with associated growth arrest, mitochondrial damage and apoptosis(18). The results from these pre-clinical studies suggest a role for targeted inhibition of FAO in addition to current treatments available or as an alternative where others fail.

A splice variant of CPT-1A which is 11 amino acids shorter at the c-terminus, has been found to be expressed exclusively in the nuclei of the MCF7 (ER-positive) cell line when compared to the MCF12F cell line (non-tumorigenic epithelial mammary cells)(19). Pucci et al, found that this variant, CPT-1Av2, forms a more stable complex with histone deacetylase-1 (HDAC1) and thus regulates genes involved in apoptosis, cell proliferation and invasion epigenetically(19, 20). HDAC1, in turn, has been implicated in mechanisms of immune evasion(21).

Taken together, these data imply that CPT-1A has a wide range of interactions with pathways involved in a range of cancer hallmarks, mediated at least in part by epigenetic mechanisms. We expected that CPT-1A would be a marker of poor outcome in breast cancer, and would show clustering with cancer hallmarks, however, the extent of these interactions has never been characterised in clinical cohorts. The aim of this analysis was to firstly characterise the predictive and/or prognostic value of CPT-1A as a biomarker at RNA and protein level using a range of online bioinformatics tools, and secondly to carry out a network analysis on the breast cancer patient cohorts available on the Oncomine platform(22) to comprehensively characterise how CPT-1A clusters globally with genes involved in all known cancer hallmarks and epigenetic pathways.

Methods

Assessment of the predictive and prognostic effect of CPT-1A expression at mRNA level was assessed using the KM plotter tool stratified by treatment and by molecular subtype. The prognostic effect at protein level was assessed using the Human Protein Atlas. Alterations in the CPT-1A genome was assessed using the cBioPortal tool(23, 24), and the prognostic effect of this was assessed using the KM plotter and G2O tools(25). The assessment of the prognostic effect of methylation of the CPT-1A gene was assessed using the MethSurv tool (26). The effect of CPT-1A on treatment response was assessed using the ROC Plotter tool.

Network analysis was performed using the Oncomine platform. The gene concepts for the cancer hallmarks, including immune regulation, were based on the NanoString concepts(27). The gene concepts for the epigenetic pathways were obtained from the EpiFactor website (28). Eight breast cancer patient cohorts had data available on the Oncomine platform (additional file 1). Clustering of the gene concept with CPT-1A was taken as significant at a $p < 0.01$ and any odds ratio. The tool specified whether the clustering occurred in the context of over or underexpression and specified the patient subgroup in which the clustering occurred. Subgroups identified in the platform included (i) subgroups related to stage, recurrence, grade and outcome, (ii) subgroups related to the histological subtype and receptor status of the tumour, and (iii) other subgroups including, but not limited to, the presence of mutations, gender, smoking status and chemotherapy resistance. The results at gene concept level, and at individual gene level for genes involved in hereditary breast cancer, were summarised and presented with further detail in the supplementary information. The raw data specifies all of the genes in the concept which cluster with CPT-1A and are available in the supplementary materials. Spearman correlations (r) to determine the relationship between CPT-1A and the immune response were obtained using the TISIDB portal(29).

Results

CPT1A expression at RNA level

Overexpression of CPT1A is a poor predictive marker at RNA level, confirmed on RNA expression data from both microarray and mRNA sequencing (Figure 1 & Table 1). High expression of CPT-1A in breast cancer patients correlates with a decreased overall survival ($p=0.007$, Figure 1a) and stage as more advanced breast cancers show higher expression of CPT-1A ($p=0.0035$, Figure 1b). Stratifying the patients by treatment status including all types of treatment showed that the CPT-1A predicted a poor outcome in treated patients only ($p=0.027$, Figure 1c and d).

Table 1

CPT-1A expression and Breast Cancer. Summary table detailing hazard ratios (HR), p values and outcomes of overall survival data in breast carcinomas showing the predictive and prognostic effects of high CPT-1A expression at mRNA and protein level, and of CPT-1A mutations/alterations. The data has been obtained from the KM plotter, G2O and the Human Protein Atlas.

Molecular level	Source	Subgroup	Method	HR	P value	Outcome
mRNA	KM plotter(30)	Treated patients	RNA microarray	1.27	0.027	Poor
mRNA	KM plotter(30)	Untreated patients	RNA microarray	1.01	0.95	NS
mRNA	KM plotter(30)	All breast carcinomas	RNA Seq	1.78	0.00042	Poor
mRNA	KM plotter(30)	Luminal A	RNA microarray	1.49	0.028	Poor
mRNA	KM plotter(30)	Luminal B	RNA microarray	1.51	0.028	Poor
mRNA	KM plotter(30)	HER2 positive	RNA microarray	1.01	0.98	NS
mRNA	KM plotter(30)	Basal phenotype	RNA microarray	1.15	0.57	NS
DNA alterations	KM plotter(30)	All breast carcinomas	DNA Seq	3.29	0.013	Poor
mRNA Metagene associated with mutation	G2O(25)	All breast carcinomas	DNA Seq and RNA microarray	0.76	8.6e-0.7	Poor
Protein	Human Protein Atlas(31)	All breast carcinoma	Immuno-histochemistry	1.80	0.00038	Poor

In addition, CPT-1A is a poor prognostic marker at protein level, confirmed on protein expression data from immunohistochemistry (p=0.00038, Table 1). The effect at RNA level was preserved when patients were split by mutation burden and the enrichment or depletion of the immune compartments (additional file 2). When patients were split by molecular subtype, the predictive effect of CPT-1A was shown to occur in luminal A and luminal B breast cancers only (p=0.028). There was no effect in HER2-positive or basal phenotype breast cancers (Table 1).

We also carried out an analysis using the ROC plotter tool(32) to investigate whether CPT-1A was a biomarker of sensitivity or resistance to endocrine therapy, HER2-directed therapy or chemotherapy in breast cancers, both in terms of complete pathological response and relapse-free survival. No significant effects were observed (additional file 3: figures 1 - 10).

Genomic alterations in CPT-1A

CPT-1A genomic alterations (mutations and copy number variations) and the metagene signature associated with CPT-1A alterations, both predicted a poor outcome. Genomic alterations in the CPT-1A gene were detected in 9% of breast cancers in the cancer genome atlas (TCGA) and METABRIC cohorts (Figure 2). Alterations in CPT-1C, the brain isoform of CPT-1, and CPT-2 which sits on the inner mitochondrial membrane, are less frequent. The majority of the genomic alterations detected in all three genes were gene amplifications (Figure 2).

Network analysis

Network analysis of the main cancer hallmarks and their associated pathways identified that CPT-1A clusters with genes in the autophagy, cell proliferation and DNA repair pathways in the context of gene overexpression (Table 2). There is also a subgroup of patients who show clustering of CPT-1A with cell proliferation in the context of underexpression. CPT-1A also clusters with genes in the angiogenesis and Hedgehog signalling pathways in the context of underexpression. High grade, poor survival and disease progression result in the association of CPT-1A with additional pathways, implying that CPT-1A recruits additional pathways as breast cancer progresses. Interestingly, the clustering of CPT-1A with JAK-STAT signalling, MAPK signalling, matrix remodelling and metastasis and Wnt signalling identifies a poor prognostic subgroup of mucinous carcinomas, a type of breast carcinoma which normally has a very good prognosis (Table 2).

In the individual gene level, CPT-1A showed clustering with wild type BRCA-1, BRCA-2, PIK3, ATM, TP53, PTEN, RAD51, EGFR, and CDH1, all moderate to high-penetrant genes known to be mutated in hereditary breast cancer (additional file 4). In addition, CPT-1A clustered with cytotoxicity genes in breast tumours showing TP53 gene mutation (Table 2).

Table 2

Summary table showing clustering of CPT-1A with genes in the listed concepts covering cancer hallmarks. The cohort in which the clustering occurred is listed. The table specifies whether the clustering occurred in the context of gene overexpression or under expression. The clustering occurred across all invasive carcinomas in the whole cohort unless otherwise specified; if clustering occurred in a specific subgroup, this is specified. Specific clustering by receptor status was only observed for HER2 positive cancers – subgroup-specific clustering did not occur for ER positive, PR positive and triple negative breast carcinomas. Advanced stage refers to overall stage unless otherwise specified. All gene clusters showing a $p < 0.01$ with any hazard ratio were taken as significant. A full breakdown of the analysis can be found in additional file 4.

Concept	Dataset	Whole cohort	Receptor Status	Dead by 5 years	High grade	Advanced stage/recurrence	Additional Subgroups
Angiogenesis	TABCHY	Underexpressed	Overexpressed - HER2 positive	No	No	No	No
Apoptosis	TABCHY	No	No	No	No	Overexpressed - N stage	No
	METABRIC	No	Overexpressed - invasive breast carcinoma (NST) – HER2 positive	Overexpressed	No	Overexpressed - tubular carcinoma	No
	METABRIC	No	No	No	Overexpressed - grade 3 lobular carcinoma	No	No
Autophagy	TABCHY	Overexpressed	No	No	No	No	No
	METABRIC	No	No	No	No	No	No
	DESMEDT	No	No	No	No	No	No
Cell proliferation	METABRIC	No	Overexpressed - HER2 positive	Overexpressed	Overexpressed - grade 3 carcinomas	No	No
	BITTNER	No	No	No	No	Overexpressed	No
	TCGA	Underexpressed and Overexpressed groups identified	No	No	No	No	No
Cytotoxicity	TABCHY	No	No	No	No	Overexpressed - N stage	No
	METABRIC	No	No	No	No	No	Overexpressed - p53 mutation
	HATZIS	No	No	No	No	Overexpressed - N stage	No
	TCGA	No	Overexpressed - HER2 positive	No	No	Overexpressed - N stage	No
DNA Damage repair	TABCHY	Overexpressed	No	No	No	No	No
Epithelial to mesenchymal transition	METABRIC	No	Overexpressed - HER2 positive	No	No	No	No
	MIYAKE	No	Overexpressed - HER2 positive	No	No	No	No
Hedghog signalling	METABRIC	Underexpressed	No	No	No	No	No
Hypoxia	METABRIC	No	No	Overexpressed	No	No	No
JAK STAT signalling	TABCHY	No	No	No	No	Overexpressed - N stage	No
	METABRIC	No	No	Underexpressed - mucinous carcinoma	Overexpressed	No	No
	HATZIS	No	No	No	No	Overexpressed - N stage	No
MAPK signalling	METABRIC	No	No	Underexpressed - mucinous carcinoma	No	No	No

Concept	Dataset	Whole cohort	Receptor Status	Dead by 5 years	High grade	Advanced stage/ recurrence	Additional Subgroups
Matrix remodelling and metastasis	METABRIC	No	No	Underexpressed - mucinous carcinoma	Overexpressed - grade 3 carcinomas	Overexpressed	No
	TCGA	No	No	No	No	No	Overexpressed - male breast cancer
Notch signalling	DESMEDT	No	No	No	No	Overexpressed - recurrence at 1 year	No
PI3K AKT signalling	TABCHY	No	No	Overexpressed	No	Underexpressed - N stage	No
TGF B signalling	TCGA	No	No	No	No	No	Underexpressed - male breast cancer
WNT signalling	METABRIC	No	No	Underexpressed - all carcinomas	No		No
				Underexpressed - mucinous carcinoma			

Epigenetic pathways

CPT-1A clusters with all of the epigenetic pathways (Table 3). Most of the pathways are recruited in invasive carcinomas without evidence of progression, with the pathways of chromatin remodelling and histone phosphorylation being additionally recruited in the context of poor survival and advanced stage. Intriguingly, histone phosphorylation clusters across all carcinomas in male but not female breast cancer (Table 3). Methylation analysis of the CPT-1A identified that methylation of CPT-1A is detected in 45 loci, of which 23 are associated with a good prognosis.

Table 3

Summary table showing clustering of CPT-1A with genes in the listed concepts covering epigenetic pathways. The cohort in which the clustering occurred is listed. The table specifies whether the clustering occurred in the context of gene overexpression or under expression. The clustering occurred across all invasive carcinomas in the whole cohort unless otherwise specified; if clustering occurred in a specific subgroup, this is specified. All gene clusters showing a $p < 0.01$ with any hazard ratio were taken as significant. BC: Breast cancer, ca: carcinoma, TN: Triple negative, PR: Progesterone receptor, ER, Estrogen receptor. A full breakdown of the analysis can be found in additional file 5.

Concept	Dataset	Whole cohort	Receptor Status	Dead by 5 years	High grade	Advanced stage	Additional Subgroups
CHROMATIN REMODELLING AND REPAIR	TCGA	No	No	No	Overexpressed	Overexpressed - invasive lobular ca	No
DNA DEMETHYLATION	TABCHY	Overexpressed	No	No	No	No	No
	METABRIC	Overexpressed	No	No	No	No	No
DNA HYDROXY-METHYLATION	METABRIC	Overexpressed	All expression levels - PR positive Underexpressed - HER2 positive	No	No	No	No
	BITTNER	Underexpressed	No	No	No	No	No
	TCGA	Overexpressed	No	No	No	No	No
DNA METHYLATION	TABCHY	Overexpressed	No	No	No	No	No
HISTONE ACETYLATION	METABRIC	No	Underexpressed - HER2 positive	No	No	No	No
HISTONE CITRULLINATION	BITTNER	Underexpressed	No	No	No	No	No
	MIYAKE	Overexpressed	No	No	No	No	No
HISTONE PHOSPHORYLATION	METABRIC	No	No	Overexpressed	No	No	No
	TCGA	No	No	No	No	Overexpressed	Overexpressed - male BC
HISTONE SUMOYLATION	DESMEDT	Underexpressed	No	No	No	No	No
	TABCHY	Overexpressed	No	No	No	No	No
HISTONE GLCNACYLATION	TABCHY	Overexpressed	No	No	No	No	No
HISTONE UBIQUITINATION	TABCHY	No	Overexpressed - TN caUnderexpressed -ER positive ca	No	No	No	No
mRNA DEGRADATION	BITTNER	All expression levels	No	No	No	No	No
mRNA EDITING	TABCHY	Overexpressed	No	No	No	No	No
	METABRIC	Overexpressed	No	No	No	No	No
TRANSCRIPTION FACTOR ACTIVATOR	TABCHY	Overexpressed	No	No	No	No	No
	BITTNER	No	No	No	No	No	Underexpressed - mucinous ca
	DESMEDT	Overexpressed	No	No	No	No	No
TRANSCRIPTION FACTOR	TABCHY	Overexpressed	No	No	No	No	No
TRANSCRIPTION FACTOR REPRESSOR	METABRIC	No	Under expressed - PR positive ca	No	No	No	No
	TABCHY	Overexpressed		No	No	No	No

Immune pathways

CPT-1A shows clustering with all of the major immune pathways (Table 4). There is a general pattern for the clustering to occur in the context of gene overexpression in carcinomas overall, and in the context of high tumour grade, poor survival and advanced stage. Clustering of CPT-1A with immune pathways in the context of underexpression occurs in tubular and mucinous carcinomas, both carcinomas which are associated with a good prognosis. Tubular carcinoma also shows clustering with the myeloid compartment in the context of overexpression (Table 4).

Increased expression of CPT-1A shows a negative correlation ($r \geq 1$, $p < 0.0001$) with abundance of tumour infiltrating lymphocytes (TILs). The strongest correlations were observed with effector memory CD4 cells, T helper 1 cells (Th1) and $T\gamma\delta$ cells, CD56bright natural killer (NK) cells, NK T cells, activated dendritic cells (DCs) and plasmacytoid DCs. Methylation also showed a strong negative correlation with $T\gamma\delta$ and activated dendritic cells but a strong positive correlation to NK cells and eosinophils. The correlation data is analytically presented in Additional file 6.

A strong negative correlation was identified between increased expression of CPT-1A and expression of immuno-inhibitory genes such as VTCN1/B7-H4 whereas a strong positive correlation was seen with KDR/VEGFR2 and IL10 expression. Increased methylation of CPT-1A was negatively associated with genes including IL10RB and positively associated with CD112 expression. No correlation was identified with the clinically relevant PD-L1 (CD274) and CTLA-4 genes (Additional file 6).

Table 4

Summary table showing clustering of CPT-1A with genes in the listed concepts covering immune pathways. The cohort in which the clustering occurred is listed. The table specifies whether the clustering occurred in the context of gene overexpression or under expression. The clustering occurred across all invasive carcinomas unless otherwise specified; if clustering occurred in a specific subgroup, this is specified. Clustering for these concepts only occurred in specific subgroups. Advanced stage refers to overall stage unless otherwise specified. All gene clusters showing a $p < 0.01$ with any hazard ratio were taken as significant. N: Nodal stage, ca: carcinoma, PR: Progesterone receptor, HER2: Human epidermal receptor 2. A full breakdown of the analysis can be found in additional file 5.

Concepts	Dataset	Receptor Status	Dead by 5 years	High grade	Advanced stage	Additional Subgroups
COSTIMULATORY SIGNALLING	TABCHY	No	No	No	Overexpressed - invasive ca	
	METABRIC	Underexpressed - PR positive Overexpressed - HER2 positive	Overexpressed	Overexpressed - grade 3	Overexpressed	
CYTOKINE AND CHEMOKINE SIGNALLING	TABCHY	Overexpressed - HER2 positive	No	No	Overexpressed - N stage	Underexpressed -mucinous ca
IMMUNE CELL ADHESION AND MIGRATION	TABCHY	No	No	No	No	Overexpressed- mixed invasive ductal and lobular carcinoma
	METABRIC	No	Overexpressed - invasive ca Underexpressed - mucinous ca	Overexpressed - grade 3 ca	Overexpressed	Under expressed -tubular ca
	HATZIS	No	No	No	Overexpressed - N stage	
INTERFERON SIGNALLING	METABRIC	No	Overexpressed	Overexpressed	No	
	TCGA	Overexpressed - HER2 positive	No	No	No	
LYMPHOID COMPARTMENT	METABRIC	Overexpressed - HER2 positive	No	Overexpressed	No	Underexpressed -tubular ca
	HATZIS	No	No	No	Overexpressed - N stage	
MYELOID COMPARTMENT	METABRIC	No	Overexpressed - invasive ca Under expressed - mucinous ca	Overexpressed	No	Overexpressed -tubular ca
	HATZIS	No	No	No	Overexpressed - N stage	
NF KAPPA B SIGNALLING	METABRIC	No	No	Overexpressed - grade 3 ca	No	

Discussion

CPT-1A is a predictive and prognostic marker of a poor outcome in luminal A and luminal B breast cancers. The effect is confirmed in different cohorts and by different methods at RNA and protein level, and also by the fact that CPT-1A identified poor prognostic subgroups in the network analysis. CPT-1A genomic alterations were detected in 9% of breast carcinomas, and are associated with a poor prognosis, as is the metagene associated with CPT-1A alterations. Moreover, methylation of CPT-1A, which would be expected to lower CPT-1A expression, is associated with a good outcome.

The network analysis revealed that CPT-1A clusters with genes involved in a wide range of cancer hallmarks, with a general pattern for CPT-1A to recruit more pathways as breast cancer progresses. The associations of CPT-1A with epigenetic and immune regulatory pathways are more extensive than was previously believed. There are also associations of CPT-1A with the wild-type variants of moderate to highly penetrant genes e.g. BRCA1, BRCA2 and ATM involved in hereditary breast cancer. Most of the associations were observed across all histological and molecular subtypes of breast cancer. In ER and PR- positive breast cancers, there was a tendency for CPT-1A to cluster in the context of underexpression. Likewise, with the good prognostic histological subtypes, tubular and mucinous carcinomas, there was a tendency for CPT-1A to cluster in the context of underexpression. A number of concepts clustered with CPT-1A in HER2-positive breast carcinoma, although CPT-1A was not identified as a predictive or prognostic biomarker in this group. The reason for this discrepancy is not clear and warrants further investigation. The association of CPT-1A with the concepts may be a consistent feature of HER2-positive breast cancer indicating the overall CPT-1A effect in cancer metabolism, conferring no additional predictive or prognostic effect in this group of patients.

In breast cancer cells, a shorter variant of CPT-1A, variant 2, has been identified in the MCF7 cell line. This variant is located in the nucleus and has been shown to interact with HDAC1 forming a more stable complex illustrating a potentially important epigenetic role(20) and identifies acetylation as the link between CPT-1A and epigenetic regulation. Our network analysis probed epigenetic links by identifying associations between CPT-1A and the genes involved in epigenetic regulation in patients. An association was found for CPT-1A with every epigenetic pathway we investigated. Unlike the concepts of the cancer hallmarks, the epigenetic pathways were mostly recruited by CPT-1A across all carcinomas regardless of the state of progression of the tumour, with only histone phosphorylation and chromatin remodelling being associated with advanced disease. These associations suggest that the epigenetic regulatory network associated with CPT-1A is more extensive and complex than previously thought. Mechanistic studies in pre-clinical models will be needed to elucidate the interactions of the network and the functional role of CPT-1A within it.

The immune landscape of breast cancer is complex. Current understanding is that CD8⁺ T cells, CD4⁺ Th1 cells, NK cells, B cells, classically activated macrophages (M1), and mature dendritic cells are hostile to tumours and participate in tumour control and elimination. On the other hand, CD4⁺ Th2 cells, regulatory B cells, CD4⁺ T regulatory cells, myeloid-derived suppressor cells, and alternatively activated macrophages (M2) are co-opted by the tumour and support tumour development and progression (33). The observation that increased CPT-1A expression and/or methylation negatively correlated with these cells suggests a possible mechanism for survival by suppressing the TILs response.

The co-option of immune cells in favour of tumour progression involves the recruitment of pro-inflammatory pathways including cytokine, chemokine, interferon and NF-kappa B signalling. It is therefore notable that CPT-1A clustered with genes in each of these concepts, identifying groups with a poor prognosis or advanced disease. Clustering of CPT-1A in costimulatory signalling, the myeloid and lymphoid compartments, and immune cell adhesion and migration also identified subgroups with a poor prognosis or disease progression. These associations suggest that the effect of CPT-1A overexpression is being mediated by a tendency to promote the co-option of the immune system and to dampen tumour control. This is supported by the finding that clustering of CPT-1A with immune concepts in the context of under expression identified histological subtypes of breast carcinoma which are associated with a favourable prognosis.

CPT-1A remained a poor prognostic marker when the components of the immune compartment were split into depleted and enriched subgroups. This indicates that the poor prognostic effect of CPT-1A is not solely dependent on immune mechanisms, a fact clearly borne out by the extent of the other associations seen. However, these data have revealed a previously unsuspected role for CPT-1A in the immune landscape of breast tumours. Recruitment of immune related pathways appears to be a late feature allowing the cancer to progress and to be an indicator of worse prognosis.

We have also observed a relationship between CPT-1A expression and expression of genes encoding for proteins targeted by immuno-inhibitors. Interestingly, a strong negative correlation is seen between CPT-1A expression and expression of VTCN1 which encodes for B7-H4; a membrane protein found on antigen presenting cells and an immune checkpoint molecule(34). It has been shown that high expression of VTCN1 correlates with a response to tyrosine kinase inhibitors in HER2-positive breast cancer(35). Increased CPT-1A expression could thus provide a mechanism of treatment resistance in these patients whereas silencing/inhibiting of CPT-1A will have a beneficial response to the patients. Further studies are needed to probe the mechanistic role CPT-1A plays in determining the balance of pro- and anti-tumour immune activity in the tumour microenvironment. It is certainly an avenue that is worth further exploration.

Studies in other cancer types support the role of CPT-1A as a driver of tumour progression. Overexpression of CPT-1A confers a worse prognosis in several cancers. In prostate cancer it has been shown that fatty acid oxidation plays an important role in cancer metabolism and that targeting CPT-1A with known inhibitors such as etomoxir leads to cell apoptosis and a reduction in cell proliferation(36). It has also been shown that in colorectal cancer, the fatty acid oxidation pathway is activated in detached cells that are able to leave the primary tumour and that high levels of CPT-1A are seen in metastatic colorectal cells. Treatment of colorectal carcinoma cells with etomoxir prevented the formation of pulmonary and hepatic metastatic nodules in mice(37). Due to its hepatotoxicity in phase II clinical trials the clinical use of etomoxir is limited(15). Teglicar has been developed in the hope of achieving a better side effect profile and has been shown to inhibit fatty acid oxidation in leukaemia cells with associated growth arrest, mitochondrial damage and apoptosis(18). Whilst a clinical use of these therapies is yet to be established, the results *in vivo* certainly give premise to further trials and studies targeting CPT-1A.

Conclusions

This large scope analysis highlights the potential of CPT-1A as a novel biomarker of breast cancer progression and to our knowledge, the first study to demonstrate the associations of CPT-1A with immune regulation. Overall, overexpression of CPT-1A confers a worse prognosis. Broad epigenetic associations are established by the time the carcinoma has become invasive and, as the cancer progresses, more pathways involved in the cancer hallmarks and the immune pathways are recruited. Although associations with cell proliferation, apoptosis and invasion have been established in other cancer types and previous studies of breast cancer, the scope of the association of CPT-1A with other hallmarks including the immune pathways and epigenetic regulation, is a new and important observation. Therapeutics that target CPT-1A, originally developed to treat heart disease and diabetes, could be repurposed in the context of breast cancer as a way to achieve sustainable tumour control and as an adjunctive treatment to immunotherapies.

Abbreviations

Carnitine palmitoyl-transferase-1 (CPT-1); World health organisation (WHO); human epidermal growth factor receptor 2 (HER2); fatty acid oxidation (FAO); etomoxir (ETO); Triple negative (TN); Progesterone receptor (PR); Estrogen receptor (ER); histone deacetylase-1 (HDAC1); tumour infiltrating lymphocytes (TILs); T helper 1 (Th1); natural killer (NK); dendritic cells (DCs); the cancer genome atlas (TCGA)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this article and its supplementary information files

The datasets supporting the conclusions of this article have been generated using the following websites:

www.kmplotter.com, <http://www.cbioportal.org>, <http://www.g-2-o.com>, <https://biit.cs.ut.ee/methsurv/>; www.rocplotter.com; <https://www.oncomine.org>, <https://epifactors.autosome.ru>; <http://cis.hku.hk/TISIDB/>

Competing interests

None declared

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Authors' contributions

Data obtained by Dr. Vijay Sharma, Mitali Das and Dr.AthinaGiannoudis. Mitali Das and Vijay Sharma have analysed the data and produced the body of the manuscript. All authors have read and approved the submitted manuscript.

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Figures

Figure 1

CPT-1A expression and clinical outcome in breast cancer.

Kaplan-Meier graphs showing difference in overall survival of breast cancer patients with high and low expression of CPT-1A (a), correlation of CPT1A expression to disease stage depicted in box plot (b) spearman: rho=0.089, p=0.00355 and differences in overall survival of treated patients (c) vs untreated patients (d).

Figure 2

Genomic alterations of the components of the carnitine shuttle in breast carcinomas from the TCGA and METABRIC cohorts.

Genomic alterations in the CPT-1A gene were detected in 9% of breast cancers. Alterations in CPT-1C and CPT-2 are less frequent. The majority of the genomic alterations detected in all three genes were gene amplifications. This dataand graph have been adopted from cBioPortal(23, 24)

Supplementary Files

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