Caveolin-1: Beyond a marker for Basal-like Breast Cancers

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Abstract

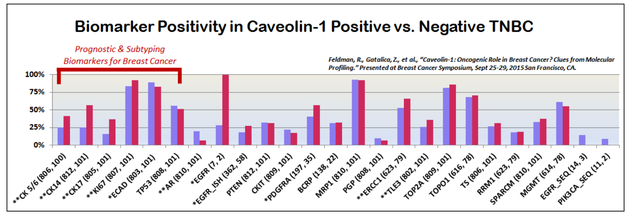
Introduction: Caveolin-1 (Cav1) is associated with basal-like triple-negative (ER-/PR-/Her2-) breast cancers (TNBC). Its biological contribution to this subtype has not been fully explored and controversy persists regarding the molecular role of Cav1 in carcinogenesis.

Experimental Procedures: Thirty-four TNBC (17 Cav1+/17 Cav1-) patients molecularly-profiled with a commercial assay (Caris Life Sciences, AZ) were evaluated retrospectively. Cav1 status was determined by immunohistochemistry (caveolin-1 polyclonal; ≥2+ ≥50%). The majority of specimens (28/34) used for profiling were from primary breast sites and contained ≥50% neoplastic cells. The transcriptomes were profiled using Illumina's HumanHT-12 microarray (v4). Data were normalized using mean normalization procedure. Differential expression analysis was performed using the 'lmFit' and 'eBayes' functions from R's Limma package.
Pathway analysis was carried out using R's signaling pathway impact analysis (SPIA) package with 69 cancer, immunity, and cell signaling related KEGG pathways.

Results: Using a cutoff of two-fold and adjusted p-value of 0.05, we identified 954 genes differentially expressed between Cav1+/- TNBC patients. Included in these were 31 genes which were found to be up-regulated by over five- fold and 3 genes down-regulated by over five fold in Cav1+ TNBC. Genes of notable interest for their role in cell signaling, cell adhesion, tumor invasion and metastasis, included an up-regulation of TGFBR2, SPARC, integrins (ITGA11, ITGB5, ITGBL1), cell adhesion proteins (LAMB3, COL5A3) and molecules which facilitate tumor invasion (LAMB3, MMP1, MMP2, MMP9). In addition, genes found to be down-regulated in Cav1+ patients and notable for their roles in promoting epithelial-mesenchymal-transition (EMT) included Claudin 3(CLD3) and CA125/MUC16 (Mucin 16). We also detected an approximately two-fold down-regulation of CDKN2A in Cav1+ patients. Using SPIA pathway analysis, 12 pathways were found to be differentially activated in Cav1+ vs. Cav1- TNBC. The most differentially activated pathways were the focal adhesion pathway (p=4.51E-18), PI3k-Akt signaling pathway (p=2.01E-6) and TGF-β and MAPK signaling pathways (p=0.005, 0.014, respectively).

Conclusions: Differential gene expression patterns and pathway analyses provide evidence for distinct profiles for gene expression between Cav1+/- TNBC. Cav1+ TNBC patients exhibit up-regulation of genes important for cell signaling, extracellular matrix remodeling and tumor invasion, and down-regulation of genes that may facilitate EMT and loss of cell cycle control. The focal adhesion pathway, as well as TGF-β, PI3K and MAPK signaling pathways, were identified as differentially activated among Cav1+/- TNBC. Taken together, these data support the role of Cav1+ in identifying a subtype of TNBC that may have a greater risk for invasion and metastasis. The correlation of this subtype with prognosis and drug response should be investigated in future studies.

Background



- Classically, CAV1 is one of several markers used to subtype basal-like breast cancers, which are characterized by low expression of ER, PR, HER2, high molecular weight cytokeratins (CK5/6, CK14, CK17) and other muscle-specific markers (laminin, integrin).
- CAV1 is a membrane protein and the main structural component of caveolae, compartments within the plasma membrane that sequester signaling molecules thus facilitating molecular "hot spots".
- Our previous data highlighted the distribution of biomarkers predictive of cytotoxic and targeted therapies in CAV1 positive and negative TNBC and demonstrated significantly higher levels of EGFR (protein, GCN), PDGFRA, ERCC1 and TLE3, but lower levels of AR in CAV1_TNBC.
- We sought to further elucidate the molecular differences in CAV1 positive and negative TNBC to identify key differences in these molecular subtypes, and distinguish a biological role for CAV1 in breast cancer pathogenesis, utilizing microarray and next-generation sequencing.

Results

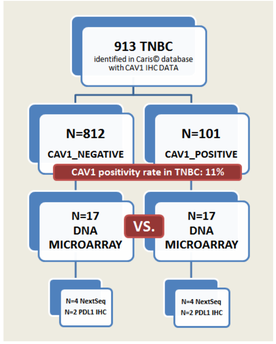


Figure 1. Selection of triple negative breast cancers with Caveolin-1 by IHC and Illumina microarray data. 592-gene hybrid-capture NGS and PDL1 IHC were performed retrospectively on selected CAV1+/- cases.

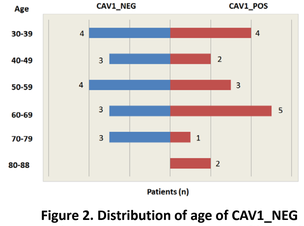


Figure 2. Distribution of age of CAV1_NEG and CAV1_POS TNBC patients profiled and included in this analysis.

Specimen Sites	n
Breast	23
Brain	2
Chest, NOS	2
Connective & Soft Tissue	2
Lymph Nodes	2
Abdomen, NOS	1
Pleura	1
Skin	1

Table 1. Specimen sites utilized for profiling included in this analysis.

Results, contd.

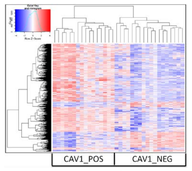


Figure 3. Heatmap of CAV1 positive/negative TNBC. Unsupervised hierarchical clustering was performed (p-value <0.05, fold change >2).

Table 2. Gene Enrichment Pathway Analysis

	Enrichment by SPIA Pathway Analysis	p-value	Status	Genes Active in Data
1	Focal adhesion	5E-18	Activated	ITGA, ITGB, FYN, SHC, Parvin, PAK, CycD
2	PI3K-Akt signaling pathway	2E-06	Activated	ITGA, ITGB, PP2A, CDK, CYCLIN, IKK, CYTOKINER
				MP, TWST, FZD, AVB5, AVB3, MMP2, MMP9, TIMP3, THBS1,
3	Proteoglycans in cancer	0.01	Inhibited	EGFR, CD63, TIMP3, LUMICAN, WNT
4	TGF-beta signaling pathway	0.01	Activated	DAN, BAMBI, BMPRII, TGFB, TGFBRII, SMAD2/3
				PARG, YAP/TAX, TGFBR, BMPRS, WNT, FZD, TCF/LEF, SLUG,
5	Hippo signaling pathway	0.01	Activated	CYCO, CTGF
6	Gap junction	0.01	Activated	CONNEXIN, TUBB, ADCY, GUCY
				PDGF, EGFR, CACN, MKP, C-FOS, PAK1/2, TNFR, TGFB, IL1,
7	MAPK signaling pathway	0.01	Activated	MKP, PPP3C, PP3CB
8	Choline metabolism in cancer	0.03	Activated	RAS, PI3K, PAP, AP1
				TGFB, TGFBR, IGF1, HOMER, EGFR, IKKA/B, SMAD3, PLK,
9	FoxO signaling pathway	0.05	Activated	CYCLIND
				CYCLIND, CDK4/6, 14-3-3-GAMMA, P14-ARF, IGF, TSP1,
10	p53 signaling pathway	0.05	Inhibited	MASPIN, SESTRINS, COP1

Table 2. Functionally enriched pathways in CAV1_POS TNBC cohort. The analysis was enriched for pathways in cancer, signal transduction pathways, cytoskeletal remodeling, cell adhesion and immune response.

Table 3. List of Differentially Expressed Genes in TNBC, CAV1_POS vs. CAV1_NEG

Gene Symbol	Role in Cancer	p-value	LogFC	Gene Symbol	Role in Cancer	p-value	LogFC
COL5A3	extracellular matrix component	1.4E-10	2.62	CLDN3	transmembrane proteins, as normal constituents of the architecture of tight junctions; marker for luminal subtypes	1.8E-06	-2.71
POSTN	matricellular protein, promotes tumor metastatic growth; plays a critical role in crosstalk between breast cancer stem cells and their niche to permit metastatic colonization	7.1E-09	2.77	CLCA2	target gene of p53 family (p53, p73 and p63), protein is induced by DNA damage in a p53-dependent manner, inhibits cell migration and invasion through suppression of the FAK signaling pathway	2.8E-06	3.2
ITGA11	part of a 7-gene signature required to initiate collective invasion in TNBC cell lines, associated with an invasive phenotype	9.2E-09	2.54	FSTL1	follistatin-like glycoprotein secreted by Snail(+) tumor cells; mediates tumor cell invasion and bone tropism, expands population of pluripotent mesenchymal cells	4.3E-06	2.45
GJA1 (CX43)	member of the connexin gene family, component of gap junctions; elevated CX43 levels have associated with improved breast cancer outcome	1.9E-08	2	MMP9	degrades basement membrane type-IV collagen; overexpression associated with aggressive phenotypes and poor prognosis; mediates proteolysis of extracellular matrix (ECM) associated with tumor invasion	0.000011	2.37
CCL14	control cell migration; associated with routine immune surveillance	2.3E-08	2.17	HAS3	Dysregulation results in abnormal production of HA and promotion of transformation and metastasis	0.00013	2.3
CDH13	loss of cadherins facilitates progression and invasion of breast cancer cells	2.6E-08	2.57	TGFBR2	ligand-binding receptor for all members of the TGF-β family; MAPK and FOXO signaling pathway	0.00015	2.01
LUM	Proteoglycans in cancer pathway - extracellular matrix proteoglycan, drives formation of a tumor-specific microenvironment	4E-08	2.64	SPRY4	SPRY4 is a potent target of the canonical WNT signaling pathway	0.00002	2.1
SLIT2	Slit2/Robo1 axis is a suppressor of breast cancer progression	8.1E-08	2.12	CDH11	epithelial-mesenchymal transition marker	0.00022	2.05
LOX	extracellular matrix modifying enzyme, has been shown to correlate with metastatic dissemination to the bone	1.9E-07	2.17	CYR61	matrix cellular proteins; YAP/Hippo pathway	0.00023	2.24
SLIT3	Slit2/Robo1 axis is a suppressor of breast cancer progression	2E-07	2.08	FAP	highly expressed in cancer-associated fibroblasts; critical role in tumorigenesis and cancer progression	0.00028	2.33
FST	down-stream target of ERR-B, mediates apoptosis, inhibitor of activin, associates with good prognosis	2.3E-07	3.13	EMP3	important role in the regulation of apoptosis, differentiation and invasion of cancer cells	0.00046	2
JAG1	metastasis effector, Notch signaling ligand	2.7E-07	2.19	PHLDA1	substrate of Aurora A, downregulation associates with breast cancer progression	0.00052	2
FHOD3	proteoglycans in ca; degradation of cell matrix	2.9E-07	2.24	LAMB3	basement membrane component	0.00057	2.64
MMP2	degrades basement membrane type-IV collagen; overexpression associated with aggressive phenotypes and poor prognosis	4.5E-07	2.16	MMP1	proteoglycans in ca (inhibits MMP2/MMP9, releases matrix-bound status)	0.00039	2.28
TNC	key molecule in tissue remodeling, high levels have been shown in invasive and metastatic cancers; down-regulator of the wnt inhibitor Dickkopf	5.1E-07	2.32	H19	proteoglycans in cancer	0.00029	2.09
PRRX1	inducer of epithelial-mesenchymal transition (EMT); silencing suppresses proliferation, migration and invasion of basal-like cancer cells, and prevents Wnt/B-catenin signaling	6.3E-07	2.18	CAMKV	HIPPO SIGNALING	0.00032	-2.02
FMO1	membrane-bound hepatic protein; involved in the metabolic activation of drugs and xenobiotic compounds,	7E-07	2.13	S100A2	secreted calcium-binding protein, when overexpressed can promote tumor metastasis	0.00082	2.13
CD209	dendritic cell function	7.8E-07	2.31	CDKN2A	tumor suppressor gene that arrests cell cycle in G1 phase inhibiting binding of CDK4/6 with cyclin D1, leaving the Rb tumor suppressor protein unphosphorylated and E2F bound and inactive	0.00088	-2.17
SNAI2 (SLUG)	master regulatory transcription factor involved in epithelial-mesenchymal transition; promotes cell invasion and tumor metastasis	1.1E-06	2.01	FLG	differentiation markers	0.001	2.14
SPARC	interacts with extracellular matrix (ECM) proteins to promote adhesion of cells from the matrix, thereby inducing a biological state conducive to cell migration; some data has shown SPARC regulates the activation of MMP-2 at the cell surface and is therefore likely to contribute to the proteolytic pathways associated with tumor invasion	1.4E-06	2.06	CALML3	may play a role in cell adhesion and migration in cancer	0.0012	2.66

Table 3. List of differentially expressed genes in CAV1_POS TNBC. Overall, 834 genes were up-regulated and 122 genes were down-regulated in CAV1_POS TNBC. Genes are listed in decreasing order of statistical significance. (down-regulated genes are highlighted in blue).

Results, contd.

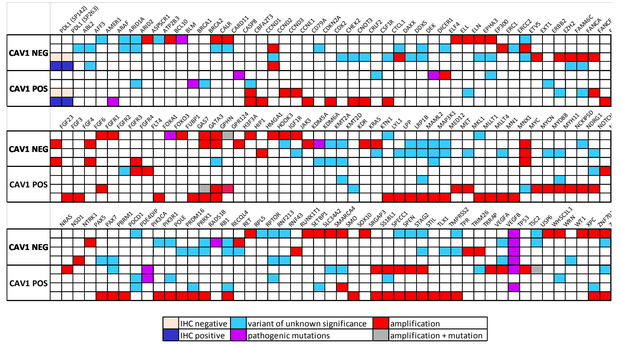


Figure 4. Genomic alterations in CAV1 POS and NEG TNBC. 592-gene hybrid-capture NGS and PDL1 IHC were performed retrospectively on selected CAV1+/- cases.

Conclusions

- Pathway analysis demonstrated several pathways that were significantly differentially activated or inhibited in CAV1_POS TNBC. The PIK3CA, TGF-β, Hippo, MAPK and FOXO signaling pathways were activated and p53 signaling pathway inhibited in CAV1_POS TNBC.
- Microarray expression analysis demonstrated 834 genes that were differentially expressed in CAV1_POS TNBC. Among these genes, most notable for their roles in facilitating cancer progression, invasion and metastasis, included extracellular matrix components(COL5A3, LUM, LOX, FHOD3, MMP2/9, LAMB3, TNC, SPARC) and epithelial-mesenchymal transition mediators (PRRX1, SLUG, CDH11). NOTCH1, YAP/HIPPO and WNT signaling mediators are among genes differentially expressed as well.
- Next-generation sequencing confirmed genomic alterations in pathways and genes observed in the pathway and expression analyses
- This data support a role of Cav1+ in identifying a subtype of TNBC that may have a greater risk for invasion and metastasis. The correlation of this subtype with prognosis and drug response should be investigated in future studies.

References

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