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Hic-5 Regulates Extracellular Matrix-associated Gene Expression and Cytokine Secretion in Cancer Associated Fibroblasts

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Abstract

The focal adhesion protein, Hic-5 plays a key role in promoting extracellular matrix deposition and remodeling by cancer associated fibroblasts within the tumor stroma to promote breast tumor cell invasion. However, whether stromal matrix gene expression is regulated by Hic-5 is still unknown. Utilizing a constitutive Hic-5 knockout, Mouse Mammary Tumor Virus-Polyoma Middle T-Antigen spontaneous breast tumor mouse model, bulk RNAseq analysis was performed on cancer associated fibroblasts isolated from Hic-5 knockout mammary tumors. Functional network analysis highlighted a key role for Hic-5 in extracellular matrix organization, with both structural matrix genes, as well as matrix remodeling genes being differentially expressed in relation to Hic-5 expression. The subcellular distribution of the MRTF-A transcription factor and expression of a subset of MRTF-A responsive genes was also impacted by Hic-5 expression. Additionally, cytokine array analysis of conditioned media from the Hic-5 and Hic-5 knockout cancer associated fibroblasts revealed that Hic-5 is important for the secretion of several key factors that are associated with matrix remodeling, angiogenesis and immune evasion. Together, these data provide further evidence of a central role for Hic-5 expression in cancer associated fibroblasts in regulating the composition and organization of the tumor stroma microenvironment to promote breast tumor progression.

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Keywords

TGFb1i1; paxillin; focal adhesions; mechanobiology; tumor stroma; breast cancer; matrix remodeling; tumor invasion; tumor angiogenesis

Introduction:

The tumor microenvironment (TME) is comprised of fibroblasts, endothelial cells, adipocytes, immune cells, as well as non-cellular components including an array of extracellular matrix (ECM) proteins, soluble cytokines and chemokines that cooperate to exert a major influence on carcinogenesis and cancer progression [1]. Cancer associated fibroblasts (CAFs) stimulated, in part, by tumor cell secreted factors, such as TGF-β, PDGF, and EGF have distinct characteristics as compared to normal fibroblasts [2] and exhibit crosstalk with both tumor cells and immune cells to promote tumor progression [3]. Additionally, CAFs themselves secrete growth factors such as HGF, FGF, PDGF and IGF to enhance tumor cell growth and survival [4, 5], as well as TGF-β and EGF that induce epithelial mesenchymal transition (EMT) in tumor cells [4, 6]. Furthermore, CAF-immune cell crosstalk is now understood to be a major source of immunosuppression in the TME [3, 7]. For example, CAF-derived TGF-β dampens the anti-tumor CD8 T cell response while promoting pro-tumorigenic Treg activity. CAFs can also influence M2 macrophage polarization by secreting cytokines such as IL-6 and CXCL12 [8–10], again leading to a suppressive environment [11]. Importantly, CAFs significantly contribute to the composition, organization and function of the stromal extracellular matrix (ECM), firstly through the secretion of structural matrix proteins, matrix crosslinking enzymes and matrix protease enzymes [12–14] and second through cell-ECM adhesion-regulated mechanical manipulation of the assembled matrix, as a result of their hypercontractile phenotype, to promote tumor invasion and migration [15]. Finally, CAFs secrete soluble cytokines to promote endothelial cell proliferation, tube formation, and the recruitment of endothelial progenitor cells, thus inducing angiogenesis to promote tumor growth [7, 16, 17].

Hic-5 (TGFβ1i1) is a focal adhesion scaffold protein that belongs to the paxillin family of LIM domain proteins [18–20]. The paxillin family of focal adhesion proteins play key roles in transducing integrin-mediated signals from the ECM to regulate cell behaviors, most notably cell migration [18, 21, 22]. Previous studies from our lab have shown that in a Mouse Mammary Tumor Virus-Polyoma Middle T-Antigen (MMTV-PyMT) breast tumor mouse model, Hic-5 plays a critical role in controlling tumor progression through CAF-dependent regulation of stromal matrix deposition and remodeling [23]. Mechanistically, Hic-5 was shown to be essential for RhoA-mediated CAF mechano-signaling to promote fibrillar adhesion formation, fibronectin fibrillogenesis and collagen fiber alignment [24], which is frequently associated with enhanced breast tumor cell invasion [25–27]. In addition, Hic-5 expression has been shown to promote the cytoplasmic-nuclear translocation of myocardin related transcription factor A (MRTF-A), to mechanically regulate myofibroblast differentiation in a TGF-β-dependent manner [28]. Hic-5 has also been shown to generate a tumor-promoting stroma by regulation of lysyl oxidase and collagen 1 in colorectal cancer [29]. Similar roles for Hic-5 have been reported in other

fibrosis-associated disorders including pancreatitis/pancreatic tumors [30], intestinal fibrosis [31] and hypertrophic scarring [32].

Herein, we have utilized Hic-5 knockout CAFs, isolated from the MMTV-PyMT breast tumor mouse model, to perform RNAseq and cytokine array analyses. We show that Hic-5 impacts the differential expression of key stromal ECM structural and regulatory genes. Our study also revealed that the production and secretion of pro-angiogenic factors and chemokines was impacted by Hic-5 expression, highlighting the role of Hic-5 in promoting a pro-tumor microenvironment in breast cancer.

Results and Discussion

Extracellular matrix-associated genes are differentially expressed in Hic-5 knockout CAFs

Previously, we reported a role for Hic-5 in cancer associated fibroblasts (CAFs) in promoting stromal matrix remodeling and breast tumor progression and invasion in a well-characterized PyMT tumor mouse model [33]. Hic-5 depletion in the Hic-5$^{-/-}$PyMT (Hic-5 KO) CAFs, within the tumor stroma resulted in diminished matrix deposition, reduced FAK signaling in the adjacent breast tumor cells and a decrease in the number of lung metastases and circulating tumor cells, while reduced tumor cell directional migration was observed in vitro in 3D extracellular matrices assembled by the Hic-5 KO CAFs [33]. To determine which genes may be regulated in association with Hic-5 expression in CAFs, Hic-5 Het and Hic-5 KO CAFs, isolated from 3 different mice, were subjected to bulk RNA sequencing analysis (RNAseq). Hic-5 Het cells were used as no significant difference was observed in tumor growth and latency between Hic-5 WT and Hic-5 Het mice in the original in vivo study [33]. A total of 48,408 genes were identified (Supp.1). Importantly, the isolated CAFs subjected to RNAseq expressed canonical fibroblast genes (VIM, ITGB1, PDGFRa, PDGFRb), but not endothelial (CD31), immune (CD45), or epithelial (EpCAM) genes (Fig. 1A and B) and western blotting confirmed expression of the CAF-specific marker alpha smooth muscle actin [34], as well as vimentin, while being negative for the epithelial marker, E-cadherin (Fig.1C). Using a filter of $p \leq 0.05$ and fold change of <-2.5 or >2.5, we identified a subset of 202 genes (Supp. 2) that were either positively or negatively regulated by Hic-5, as depicted in the Volcano plot (Fig 1A) and the heatmap depicting the unsupervised clustering of differentially expressed genes (Fig 1D).

KEGG pathway analysis was performed using Partek Flow software and a total of 21 pathways were identified with an enrichment score higher than three (Fig 1E). Notably, focal adhesions and the TGF-β signaling pathway were enriched by Hic-5 expression, consistent with its previously recognized functions in mechano-signaling and TGF-β signaling and regulation [24, 32, 35]. The other enriched pathways are sub-grouped into key pathways in cancer, immune activation associated, pathways in different cancer and cardiomuscular-associated pathways (Fig 1E). Gene ontology (GO) analysis revealed that extracellular matrix (ECM)-associated terms were highly enriched, including ECM genes, external encapsulating structure, extracellular region and ECM organization (Fig 1F).

Hic-5-regulated ECM genes are involved in various cancers

A closer examination of the ECM-associated genes that were differentially regulated by Hic-5 identified key structural matrix genes, as well as matrix remodeling genes (Fig 2A). Regulated matrix genes included the fibrillar collagen genes Col17a1, Col18a1, Col28a1, Col5a2 and Col9a3, proteoglycans/glycoproteins genes FMOD, MFAP5, SPARC, TNC, NID2 and OPTC, ECM anchoring genes PRELP, ABI3BP, NID2 and OPTC, ECM remodeling genes MMP9, MMP23, CELA1, ADAMTS5, CPZ and HPSE, and soluble proteins including cytokines and growth factors that are typically enriched in the tumor stroma, IGF1, CLEC3B, PTX3, SVEP1 and SFRP1 (Fig 2A). A list of the respective gene's full names is included in Table 1.

STRING analysis was next performed to identify experimentally-determined and computer-predicted protein-protein interactions amongst the Hic-5-regulated gene products. The network nodes represent a total of twenty-four ECM-associated genes, and each edge represents an interaction (Fig 2B). Col5a2 is central to the interactome of all five collagen isoforms (Fig 2B). It is a type V collagen that is associated with poor clinical outcome in multiple cancers including colorectal cancer [36], gastric cancer [37], bladder cancer [38] and prostate cancer [39]. SPARC, also known as osteonectin or BM-40, is another key ECM gene with multiple interactions with collagen Col5a2, Col9a3 and Col18a1, MMP9, proteoglycan FMOD, ECM anchoring protein NID2 and the chemokine IGF1. SPARC is a secreted glycoprotein that is important for collagen matrix assembly during mammalian development [40]. The capacity for SPARC to promote or inhibit cancer progression is context dependent. SPARC expression has been reported to have a positive correlation with bladder carcinoma [41], osteosarcoma [42], breast carcinoma [43], colorectal cancer [44], head and neck squamous cell carcinoma [45], lung squamous cell carcinoma [46], prostate carcinoma [47], gastric cancer [48] and melanoma [49]. Conversely, SPARC exhibited a tumor suppressor role in ovarian cancer by inhibiting metastasis [50].

Functional enrichment analysis using the STRING database identified over a hundred publications that were directly linked to the Hic-5-dependent ECM gene network (Supp. 3), identifying five functional categories into which the ECM genes were distributed, namely matrix assembly, fibrosis, TGF-β signaling, cancer and angiogenesis (Fig 2C). We determined that MMP9, Col18a1, FMOD, PTX3, ADAMTS5, IGF1 and TNC are associated with all five functionalities, whereas Col28a1, Col9a3, CPZ, CELA1, PRELP and OPTC were only associated with matrix assembly. The other genes were associated with at least two functions (Fig 2C). For the seven genes that were linked to most functionalities, their products are extracellular matrix proteins such as collagens and tenascin C (TNC), matrix proteases ADAMTS5, MMP9, the proteoglycan FMOD and soluble factors that regulate an immune response (PTX3) or cell growth (IGF1).

Fibromodulin (FMOD) is a proteoglycan that modulates collagen fibrillogenesis through interaction with the collagen cross-linking enzyme lysyl oxidase (LOX), therefore contributing to collagen fiber bundling and alignment of this major stromal ECM component. This aspect of fibrillar collagen remodeling is a key factor in promoting tumor invasion in human breast cancer patients [25, 27]. Importantly, Hic-5 KO CAFs are defective in assembling a highly ordered collagen/fibronectin matrix, in association with reduced

tumor cell signaling and invasion, both *in vivo* and *in vitro* [33]. The precise role of FMOD in cancer varies depending on cancer type and model system. For example, FMOD is up-regulated in chronic lymphocytic leukemia, mantle cell lymphoma, glioblastoma, prostate cancer and myxoma in human samples, whereas in a colon cancer mouse model and a small cell lung cancer cell line, FMOD was down regulated [51]. Secreted FMOD promotes cell migration by activating focal adhesion-associated integrin-FAK-Src-Rho GTPase signaling that results in remodeling of the actin cytoskeleton organization to promote cell migration [52]. FMOD is also involved in tumor angiogenesis through regulation of the expression of angiopoietin 2 (ANG2) and VEGF [53, 54], as recently demonstrated in gliomas [52]. Prolargin (PRELP) is structurally related to FMOD, and the STRING analysis indicates that both genes and protein of PRELP and FMOD can be co-expressed (Fig 2B). Accordingly, PRELP also serves as an ECM anchoring protein that connects the fibroblast cell surface to the ECM to enhance focal adhesion formation [55, 56].

CLEC3 is a calcium binding protein that localizes in the cytoplasm and extracellular matrix. Interestingly, although CLEC3 expression was reduced in Hic-5 KO CAFs, it does not have functional connections with the other differentially regulated genes in the STRING analysis (Fig 2B). Nevertheless, it is highly expressed in fibroblasts [57], and like Hic-5 is involved in the cellular response to TGF-β and is associated with tumor invasion, metastasis and extracellular proteolysis [58].

MMP23 is one of the less studied MMPs that and was downregulated in Hic-5 KO CAFs (Fig 2A). MMP23 was first documented as CA-MMP for its distinct cysteine array motif back in 1999 [59]. MMP-23 is involved in intracellular trafficking of potassium channels, and co-expression of MMP-23 and potassium channel KV1.3 is associated with colorectal cancers [60]. On the other hand, in human melanoma, MMP23 expression is correlated with recurrence in immune therapy patients and associated with worse outcome, as MMP-23 dampens T cell activity through cleavage of cytokine and chemokine regulatory proteins [61, 62].

The extracellular matrix proteases, MMP9 and ADAMTS5 have well-documented roles in the remodeling of the ECM and in promoting angiogenesis [63–67]. However, the role of MMP9 in pro-/anti-cancer progression is controversial. MMP9 is known to positively relate to cancer invasion, metastasis, and angiogenesis [63] and has been used as a predictor of poor clinical outcome in some cancers [68]. Meanwhile, high expression of MMP9, in contrast to MMP2, was found to be associated with a more favorable prognosis in cervical cancer [69]. MMP9 can also function as a potent regulator for the innate immune response and thus perform an anti-tumor role [70]. Therefore, MMP23 was downregulated in Hic-5 KO CAFs, while MMP9 was upregulated in this population, indicating that Hic-5 likely exerts a complex, bi-modal role in controlling ECM remodeling by this protease family in the tumor microenvironment.

Hic-5 regulates cytokine production in CAFs

In addition to their role in ECM deposition and remodeling within the tumor stroma, CAFs also secrete soluble factors and release membrane-bound microvesicles/exosomes, carrying miRNAs and proteins into the TME to influence tumor cell signaling, tumor angiogenesis,

as well as localized immune cell infiltration and activation [4, 71–73]. Hic-5 has previously been shown to regulate a TGF-β autocrine loop in myofibroblasts, suggesting that Hic-5 may also regulate growth factor/cytokine secretion in CAFs [32]. Since we also detected changes in the mRNA levels of several cytokines in the Hic-5 KO CAFs (Fig 2A), we performed a mouse cytokine array assay, using conditioned media from CAFs to identify secreted cytokines that were regulated by Hic-5 expression. A total of ten secreted cytokines were detected in two independent experiments (Fig 2D and E and Supp. 4).

Interestingly, neither Hic-5 Het and KO CAFs secrete detectable levels of most of the chemokine (C-X-C motif) ligands (CXCL), C-C motif chemokine ligands (CCL) or interleukins. However, the Hic-5 KO cells secreted reduced levels of the chemokine, CCL17 (Fig 2D and supp. 4). CCL17, also known as thymus and activation regulated chemokine (TARC), was the first identified T-cell chemoattractant CC chemokine, and is constitutively produced in the thymus [74]. In the TME, CCL17 secreted by cancer associated fibroblasts and neutrophils [75, 76] plays anti-tumor roles by recruiting immunosuppressive regulatory T cells [77, 78]. Thus, the impaired secretion of CCL17 by Hic-5 KO CAFs suggests a pro-tumor role for Hic-5 within the tumor stroma, by regulating the crosstalk between CAFs and immune cells.

Pentraxin-3 (PTX3), which showed a large fold change reduction in the RNAseq analysis, was also reduced in the Hic-5 KO CAF conditioned media, as compared to the Hic-5 Het samples (Fig 2E). PTX3 is well-known for its role in innate immunity and inflammation [79]. It is also involved in endothelial cell dysfunction through various mechanisms. For example, PTX3 binds to fibroblast growth factor-2 (FGF2), thereby inhibiting FGF2 binding to endothelial cell receptors. This PTX3/FGF2 interaction is capable of suppressing angiogenesis in nude mice [80]. Conversely, PTX3 was shown to promote angiogenesis after stroke in a PTX3 knockout mouse model [81, 82].

MMP2 is another important matrix-targeting protease that is frequently implicated in remodeling of the tumor microenvironment to promote tumor cell invasion [83]. Our RNAseq analysis revealed that MMP2 was downregulated in Hic-5 KO CAFs, but did not reach statistical significance (p value 0.07, Fold change −2.53). Interestingly, MMP2 is also implicated in angiogenesis. Direct interaction of MMP2 with αvβ3 integrin in blood vessels promotes endothelial cell proliferation and survival, as well as cell invasion by remodeling the surrounding matrix [84]. MMP2 is also involved in VEGF-mediated angiogenesis in lung cancer, as MMP2 RNA interference disrupted VEGF-dependent endothelial tube formation [85]. In contrast, the level of IGFBP-6, which is involved in cell growth and cell survival, was secreted at similar levels by both the Hic-5 Het and the KO CAFs (Fig 2E).

The decreased levels of secreted Pentraxin-3, MMP2 and VEGF from the Hic-5 KO CAFs, combined with the down-regulation of ECM genes like FMOD, further suggest an important role for Hic-5 promoting tumor angiogenesis through CAF-endothelial cell crosstalk (Fig 2E). [86].

Hic-5 modulates MRTF-A activity to indirectly regulate gene expression

Although there is no current evidence that Hic-5 functions as a transcription factor, it has been shown that Hic-5 acts as a co-regulator in nuclear receptor (glucocorticoid and androgen receptor) and transcription factor (SPI1, SMADs) mediated gene expression [87–90]. Additionally, Hic-5 can exert gene expression regulation through activation of other transcription factors, such as MRTF-A, by increasing the cell-ECM mechanosignaling and associated remodeling of the actin cytoskeleton, to promote MRTF-A nuclear translocation [28]. Therefore, we sought to examine whether Hic-5 may be regulating some of the cytoskeletal remodeling genes though modulating MRTF-A activity in the CAFs [91]. Indeed, when the cells were seeded overnight on a fibronectin-coated substrate, Hic-5 KO CAFs displayed increased cytoplasmic and decreased nuclear MRTF-A localization compared to Hic-5 Het CAFs (Fig 3A and B), suggesting impaired activity of MRTF-A in the absence of Hic-5 expression. Furthermore, the RNAseq data demonstrated that a subset of MRTF-A response genes were downregulated in the Hic-5 KO CAFs (Fig 3C), suggesting that Hic-5 can indirectly regulate CAF gene expression through mechano-signaling from focal adhesions.

In summary, we have performed RNAseq and cytokine array analysis on PyMT breast tumor-derived Hic-5 Het and KO CAFs. We have identified a cadre of ECM-associated genes and cytokines that are regulated by Hic-5 expression and play significant tumor-promoting roles in the stroma of the TME, including the deposition and remodeling of the ECM and the stimulation of tumor angiogenesis. These gene-regulatory functions of Hic-5, including its regulation of MRTF-A transcription factor localization, likely complement its previously reported role in focal adhesion mechanobiology to promote stromal ECM remodeling and breast tumor cell invasion [21, 24, 33], and in other fibrotic disorders such as pancreatitis/pancreatic cancer [30] and hypertrophic scarring [28, 29, 32, 92].

Material and Methods:

CAF isolation and culture

Cancer associated fibroblasts (CAFs) were isolated from Hic-5 Het and Hic-5 KO MMTV-PyMT tumor bearing mice, as previously described [33]. Briefly, tumors were minced and digested in digestion media (2mg/mL collagenase, 2mg/mL trypsin in 50:50 DMEM:F12, 5% FBS, 5μg/mL Insulin and 10 I.U. penicillin/10 μg/mL streptomycin) for 50 minutes at 37°C. Differential centrifugation was performed to separate the single CAFs from tumor organoids. CAFs were cultured and expanded in vitro using PyMT media (50:50 DMEM:F12 with 10% FBS, 2 mM L-glutamine and 10 I.U. penicillin/10 μg/mL streptomycin), 5% CO_2 and 37°C.

High throughput RNA-sequencing and gene-set analysis

Whole transcriptome profiling was performed on biological triplicates on Hic-5 Het and Hic-5 KO CAFs at the SUNY Molecular Analysis Core (SUNYMAC) facility at Upstate Medical University. RNA was isolated using Trizol reagent from confluent plates of ex vivo expanded cells. RNA quality and quantity were assessed using the RNA 6000 Nano Kit on the Agilent Bioanalyzer 2100. Sequencing libraries were prepared with the TruSeq Stranded

Total RNA Library Prep Kit RiboZero Gold (Illumina: San Diego, CA), using 1ug of total RNA as input. Library size was assessed with the DNA 1000 Kit on the Agilent Bioanalyzer 2100, and libraries were quantified with the Qubit dsDNA HS Assay Kit (Invitrogen: Waltham, MA USA). Libraries were pooled and sequenced on the NextSeq 500 instrument (Illumina: San Diego, CA), with a single end 1×75bp read using a High Output 150 cycle reagent kit. Fastq files were trimmed to remove adapter sequences using Cutadapt version 1.2.1 and were aligned using Bowtie2 version 2.2.5 to database Ensemble Transcripts release 86. Quantify to annotation modeling was performed via Partek E/M. Data was normalized by reads per kilobase per million (RPKM). Differential gene expression analyses were performed in Partek Flow using GSA task. The raw RNAseq data have been submitted to the NCBI GEO and the accession number is: GSE211898)

Mouse cytokine array analysis

Cytokine arrays were performed in duplicate according to the manufacturer's instructions (Mouse XL Cytokine Array Kit, Catalog # ARY028, R&D Systems: Minneapolis, MN USA). Briefly, confluent CAF cultures were grown in complete PyMT media. Once confluent, the media was changed to serum free PyMT media and incubated for 24 hours. The conditioned media was collected, filtered and incubated on the membranes overnight at 4°C with rocking, according to the manufacturer's recommendations. The membranes were incubated with the detection antibody cocktail for 1 hour at RT and Streptavidin-HRP for 30 minutes at RT with extensive washing in between. The Hic-5 Het and KO membranes were then developed using the Chemi-reagent mix and imaged simultaneously on a Bio Rad Chemiluminescent Imager. Mean pixel density was calculated from each spot and the fold change was calculated by dividing the densities from the average of the control densities (Hic-5 Het).

Immunofluorescence microscopy and western blotting

CAFs on FN-coated glass coverslips were fixed with 4% PFA in PBS for 15 min, permeabilized with 1% Triton X-100 in PBS for 15 min and blocked with 3% bovine serum albumin (BSA) for 1 h at room temperature (RT). Coverslips were then stained with MRTF-A (1:100; sc-390324, SANTA CRUZ Biotechnology) primary antibody diluted in 3% BSA for 2 hours at 37°C, followed by incubation in DyLight 550-conjugated goat anti-Mouse secondary antibody (1:400, 84540; Thermo Fisher) for 1 hours at RT. The coverslips were washed in PBS + 0.1% Triton X-100, and mounted. CAFs were imaged using a Zeiss Axioskop2 plus microscope fitted with a Q imaging EXi Blue CCD camera using a Plan-Apochromat 40X/0.75 NA objective. Antibodies used for western blotting were alpha smooth muscle actin (1:1000; A2547 Sigma Aldrich), Hic-5 (1:800; 611165. BD Biosciences), vimentin (1:1000; 5741s Cell Signaling), E-cadherin (1:1000; 3195 Cell Signaling) and GAPDH (1:1000; 60004–1-Ig Proteintech).

Statistical Analysis

All data were analyzed using a two-sided t-test using Microsoft excel or GraphPad Prism. Statistical significance is indicated by *P<0.05, **P<0.01, ***P<0.005. The data are mean ± s.e.m. or SD as denoted in the figure legend.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations:

CAFs	Cancer Associated Fibroblasts
CLEC3	C-type lectin domain family 3
CCL17	C-C Motif Chemokine Ligand 17
CD31	Cluster of Differentiation 31
CD45	Cluster of Differentiation 45
CXCL	Chemokine (C-X-C) Motif Ligand
ECM	Extracellular Matrix
EGF	Epithelial Growth Factor
EMT	Epithelial Mesenchymal Transition
EpCAM	Epithelial Cell Adhesion Molecule
FGF	Fibroblast Growth Factor
FMOD	Fibromodulin
GO	Gene Ontology
Hic-5	Hydrogen Peroxide Inducible Clone 5
HGF	Hepatocyte Growth Factor
IGF	Insulin Growth Factor
IL6	Interleukin 6
ITGB1	Integrin Beta 1
LOX	Lysyl Oxidase
MMTV-PyMT	Mouse Mammary Tumor Virus-Polyoma Middle T-Antigen
MMP2	Matrix Metallopeptidase 2
MMP23	Matrix Metallopeptidase 23

MMP9	Matrix Metallopeptidase 9
MRTF-A	Myocardin Related Transcription Factor A
PDGF	Platelet Derived Growth Factor
PDGFRa	Platelet Derived Growth Factor Receptor Alpha
PDGFRb	Platelet Derived Growth Factor Receptor Beta
PRELP	Prolargin
PTX3	Pentraxin 3
RPKM	Reads per kilobase per million
SPARC	Secreted Protein Acidic and Cysteine Rich
TARC	Thymus and Activation Regulated Chemokine
TGF-β	Transforming Growth Factor Beta
TGFB1i1	Transforming Growth Factor Beta 1 Induced Transcript 1
TME	Tumor Microenvironment
TPM	Transcripts per million
TNC	Tenascin C
VEGF	Vascular Endothelial Growth Factor
VIM	Vimentin

Reference:

1. Soysal SD, Tzankov SE Muenst A Fau -, and Muenst SE, Role of the Tumor Microenvironment in Breast Cancer. (1423–0291 (Electronic)).
2. Mao Y, et al. , Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev, 2013. 32(1–2): p. 303–15. [PubMed: 23114846]
3. Barrett RA-O and Puré EA-O, Cancer-associated fibroblasts and their influence on tumor immunity and immunotherapy. LID - 10.7554/eLife.57243 [doi] LID - e57243. (2050–084X (Electronic)).
4. Sahai E, et al. , A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer, 2020. 20(3): p. 174–186. [PubMed: 31980749]
5. Kalluri R, The biology and function of fibroblasts in cancer. Nat Rev Cancer, 2016. 16(9): p. 582–98. [PubMed: 27550820]
6. Cook DP and Vanderhyden BC, Context specificity of the EMT transcriptional response. Nat Commun, 2020. 11(1): p. 2142. [PubMed: 32358524]
7. Lazennec G and Richmond A, Chemokines and chemokine receptors: new insights into cancer-related inflammation. (1471–499X (Electronic)).
8. Biffi G, et al., IL1-Induced JAK/STAT Signaling Is Antagonized by TGFβ to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma. (2159–8290 (Electronic)).
9. Öhlund DA-O, et al., Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. (1540–9538 (Electronic)).

10. Elyada E, et al., Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. (2159–8290 (Electronic)).
11. Chen S, et al. , Cancer-associated fibroblast-induced M2-polarized macrophages promote hepatocellular carcinoma progression via the plasminogen activator inhibitor-1 pathway. LID - 59 [pii] LID - 10.3892/ijo.2021.5239 [doi]. (1791–2423 (Electronic)).
12. Nguyen EV, et al. , Proteomic Profiling of Human Prostate Cancer-associated Fibroblasts (CAF) Reveals LOXL2-dependent Regulation of the Tumor Microenvironment. Mol Cell Proteomics, 2019. 18(7): p. 1410–1427. [PubMed: 31061140]
13. Maller O, DuFort CC, and Weaver VM, YAP forces fibroblasts to feel the tension. Nat Cell Biol, 2013. 15(6): p. 570–2. [PubMed: 23728464]
14. Rosenthal EL, et al. , Expression of proteolytic enzymes in head and neck cancer-associated fibroblasts. Arch Otolaryngol Head Neck Surg, 2004. 130(8): p. 943–7. [PubMed: 15313864]
15. Karagiannis GS, et al. , Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol Cancer Res, 2012. 10(11): p. 1403–18. [PubMed: 23024188]
16. Carmeliet P, VEGF as a key mediator of angiogenesis in cancer. Oncology, 2005. 69 Suppl 3: p. 4–10. [PubMed: 16301830]
17. Orimo A, et al. , Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell, 2005. 121(3): p. 335–48. [PubMed: 15882617]
18. Deakin NO and Turner CE, Paxillin comes of age. J Cell Sci, 2008. 121(Pt 15): p. 2435–44. [PubMed: 18650496]
19. Turner CE, Paxillin and focal adhesion signalling. Nat Cell Biol, 2000. 2(12): p. E231–6. [PubMed: 11146675]
20. Thomas SM, Hagel M, and Turner CE, Characterization of a focal adhesion protein, Hic-5, that shares extensive homology with paxillin. J Cell Sci, 1999. 112 (Pt 2): p. 181–90. [PubMed: 9858471]
21. Alpha KM, Xu W, and Turner CE, Paxillin family of focal adhesion adaptor proteins and regulation of cancer cell invasion. Int Rev Cell Mol Biol, 2020. 355: p. 1–52. [PubMed: 32859368]
22. Deakin NO, Pignatelli J, and Turner CE, Diverse roles for the paxillin family of proteins in cancer. Genes Cancer, 2012. 3(5–6): p. 362–70. [PubMed: 23226574]
23. Goreczny GJ, et al., Hic-5 remodeling of the stromal matrix promotes breast tumor progression. (1476–5594 (Electronic)).
24. Goreczny GJ, Forsythe IJ, and Turner CE, Hic-5 regulates fibrillar adhesion formation to control tumor extracellular matrix remodeling through interaction with tensin1. (1476–5594 (Electronic)).
25. Pickup MW, Mouw JK, and Weaver VM, The extracellular matrix modulates the hallmarks of cancer. EMBO Rep, 2014. 15(12): p. 1243–53. [PubMed: 25381661]
26. Mouw JK, Ou G, and Weaver VM, Extracellular matrix assembly: a multiscale deconstruction. Nat Rev Mol Cell Biol, 2014. 15(12): p. 771–85. [PubMed: 25370693]
27. Gehler S, et al. , Bi-directional signaling: extracellular matrix and integrin regulation of breast tumor progression. Crit Rev Eukaryot Gene Expr, 2013. 23(2): p. 139–57. [PubMed: 23582036]
28. Varney SD, et al. , Hic-5 is required for myofibroblast differentiation by regulating mechanically dependent MRTF-A nuclear accumulation. J Cell Sci, 2016. 129(4): p. 774–87. [PubMed: 26759173]
29. Omoto T, et al. , The impact of stromal Hic-5 on the tumorigenesis of colorectal cancer through lysyl oxidase induction and stromal remodeling. Oncogene, 2018. 37(9): p. 1205–1219. [PubMed: 29242607]
30. Gao L, et al. , Hic-5 is required for activation of pancreatic stellate cells and development of pancreatic fibrosis in chronic pancreatitis. Sci Rep, 2020. 10(1): p. 19105. [PubMed: 33154390]
31. Paul J, et al. , IL-17-driven intestinal fibrosis is inhibited by Itch-mediated ubiquitination of HIC-5. Mucosal Immunol, 2018. 11(2): p. 427–436. [PubMed: 28612841]
32. Dabiri G, et al. , Hic-5 promotes the hypertrophic scar myofibroblast phenotype by regulating the TGF-beta1 autocrine loop. J Invest Dermatol, 2008. 128(10): p. 2518–25. [PubMed: 18401422]

33. Goreczny GJ, et al. , Hic-5 remodeling of the stromal matrix promotes breast tumor progression. Oncogene, 2017. 36(19): p. 2693–2703. [PubMed: 27893716]
34. Nurmik M, et al. , In search of definitions: Cancer-associated fibroblasts and their markers. Int J Cancer, 2020. 146(4): p. 895–905. [PubMed: 30734283]
35. Pignatelli J, et al. , Hic-5 promotes invadopodia formation and invasion during TGF-β-induced epithelial-mesenchymal transition. J Cell Biol, 2012. 197(3): p. 421–37. [PubMed: 22529104]
36. Wang JA-O, et al., Increased Collagen Type V α2 (COL5A2) in Colorectal Cancer is Associated with Poor Prognosis and Tumor Progression. (1178–6930 (Print)).
37. Brodsky AA-O, et al., Somatic mutations in collagens are associated with a distinct tumor environment and overall survival in gastric cancer. (1471–2407 (Electronic)).
38. Ingenwerth M, et al. , The prognostic value of cytokeratin and extracellular collagen expression in urinary bladder cancer. LID - 10.2174/1566524021666210225100041 [doi]. (1875–5666 (Electronic)).
39. Ren X, et al., COL5A2 Promotes Proliferation and Invasion in Prostate Cancer and Is One of Seven Gleason-Related Genes That Predict Recurrence-Free Survival. (2234–943X (Print)).
40. Arnold SA and Brekken RA, SPARC: a matricellular regulator of tumorigenesis. (1873–961X (Electronic)).
41. Nimphius W, et al., CD34+ fibrocytes in chronic cystitis and noninvasive and invasive urothelial carcinomas of the urinary bladder. (0945–6317 (Print)).
42. Dalla-Torre CA, et al., Effects of THBS3, SPARC and SPP1 expression on biological behavior and survival in patients with osteosarcoma. (1471–2407 (Electronic)).
43. Shi S, et al., Prognostic Significance of SPARC Expression in Breast Cancer: A Meta-Analysis and Bioinformatics Analysis. (2314–6141 (Electronic)).
44. Kaiser S, et al., Transcriptional recapitulation and subversion of embryonic colon development by mouse colon tumor models and human colon cancer. (1474–760X (Electronic)).
45. Kato Y, et al., Expression of SPARC in tongue carcinoma of stage II is associated with poor prognosis: an immunohistochemical study of 86 cases. (1107–3756 (Print)).
46. Koukourakis MI, et al., Enhanced expression of SPARC/osteonectin in the tumor-associated stroma of non-small cell lung cancer is correlated with markers of hypoxia/acidity and with poor prognosis of patients. (0008–5472 (Print)).
47. Thomas R, et al., Differential expression of osteonectin/SPARC during human prostate cancer progression. (1078–0432 (Print)).
48. Maeng HY, et al., Osteonectin-expressing cells in human stomach cancer and their possible clinical significance. (0304–3835 (Print)).
49. Massi D, et al., Osteonectin expression correlates with clinical outcome in thin cutaneous malignant melanomas. (0046–8177 (Print)).
50. John B, et al., Regulation of the bi-directional cross-talk between ovarian cancer cells and adipocytes by SPARC. (1476–5594 (Electronic)).
51. Pourhanifeh MH, et al., The role of fibromodulin in cancer pathogenesis: implications for diagnosis and therapy. (1475–2867 (Print)).
52. Mondal B, et al. , Integrative functional genomic analysis identifies epigenetically regulated fibromodulin as an essential gene for glioma cell migration. Oncogene, 2017. 36(1): p. 71–83. [PubMed: 27212030]
53. Ao Z, et al. , Tumor angiogenesis of SCLC inhibited by decreased expression of FMOD via downregulating angiogenic factors of endothelial cells. Biomed Pharmacother, 2017. 87: p. 539–547. [PubMed: 28081464]
54. Adini I, et al. , Melanocyte-secreted fibromodulin promotes an angiogenic microenvironment. J Clin Invest, 2014. 124(1): p. 425–36. [PubMed: 24355922]
55. Bengtsson E, et al., The leucine-rich repeat protein PRELP binds fibroblast cell-surface proteoglycans and enhances focal adhesion formation. (1470–8728 (Electronic)).
56. Mikaelsson E, et al. , A proline/arginine-rich end leucine-rich repeat protein (PRELP) variant is uniquely expressed in chronic lymphocytic leukemia cells. PLoS One, 2013. 8(6): p. e67601. [PubMed: 23826326]

57. Xie T, et al., Single-Cell Deconvolution of Fibroblast Heterogeneity in Mouse Pulmonary Fibrosis. (2211–1247 (Electronic)).
58. Xie XW, Jiang SS, and Li X, CLEC3B as a Potential Prognostic Biomarker in Hepatocellular Carcinoma. (2296–889X (Print)).
59. Pei D, CA-MMP: a matrix metalloproteinase with a novel cysteine array, but without the classic cysteine switch. FEBS Lett, 1999. 457(2): p. 262–70. [PubMed: 10471791]
60. Nguyen HM, et al. , Intracellular trafficking of the KV1.3 potassium channel is regulated by the prodomain of a matrix metalloprotease. J Biol Chem, 2013. 288(9): p. 6451–64. [PubMed: 23300077]
61. Moogk D, et al. , Melanoma expression of matrix metalloproteinase-23 is associated with blunted tumor immunity and poor responses to immunotherapy. J Transl Med, 2014. 12: p. 342. [PubMed: 25491880]
62. Galea CA, et al. , Domain structure and function of matrix metalloprotease 23 (MMP23): role in potassium channel trafficking. Cell Mol Life Sci, 2014. 71(7): p. 1191–210. [PubMed: 23912897]
63. Quintero-Fabián S, et al., Role of Matrix Metalloproteinases in Angiogenesis and Cancer. (2234–943X (Print)).
64. Mira E, et al. , Secreted MMP9 promotes angiogenesis more efficiently than constitutive active MMP9 bound to the tumor cell surface. J Cell Sci, 2004. 117(Pt 9): p. 1847–57. [PubMed: 15075244]
65. Rafii S, et al. , Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nat Rev Cancer, 2002. 2(11): p. 826–35. [PubMed: 12415253]
66. Bergers G, et al. , Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol, 2000. 2(10): p. 737–44. [PubMed: 11025665]
67. Kumar S, Rao N, and Ge R, Emerging Roles of ADAMTSs in Angiogenesis and Cancer. Cancers (Basel), 2012. 4(4): p. 1252–99. [PubMed: 24213506]
68. Joseph CA-O, et al., Elevated MMP9 expression in breast cancer is a predictor of shorter patient survival. (1573–7217 (Electronic)).
69. Azevedo Martins JM, et al., Tumoral and stromal expression of MMP-2, MMP-9, MMP-14, TIMP-1, TIMP-2, and VEGF-A in cervical cancer patient survival: a competing risk analysis. (1471–2407 (Electronic)).
70. Leifler KS, et al., Inflammation induced by MMP-9 enhances tumor regression of experimental breast cancer. (1550–6606 (Electronic)).
71. Kaur A, et al. , Remodeling of the Collagen Matrix in Aging Skin Promotes Melanoma Metastasis and Affects Immune Cell Motility. Cancer Discov, 2019. 9(1): p. 64–81. [PubMed: 30279173]
72. Bhome R, et al. , Exosomal microRNAs derived from colorectal cancer-associated fibroblasts: role in driving cancer progression. Aging (Albany NY), 2017. 9(12): p. 2666–2694. [PubMed: 29283887]
73. Fearon DT, The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. Cancer Immunol Res, 2014. 2(3): p. 187–93. [PubMed: 24778314]
74. Imai T, et al. , Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. J Biol Chem, 1996. 271(35): p. 21514–21. [PubMed: 8702936]
75. Mishalian I, et al. , Neutrophils recruit regulatory T-cells into tumors via secretion of CCL17--a new mechanism of impaired antitumor immunity. Int J Cancer, 2014. 135(5): p. 1178–86. [PubMed: 24501019]
76. Omland SH, et al. , Cancer associated fibroblasts (CAFs) are activated in cutaneous basal cell carcinoma and in the peritumoural skin. BMC Cancer, 2017. 17(1): p. 675. [PubMed: 28987144]
77. Mizukami Y, et al. , CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of Foxp3+ regulatory T cells in gastric cancer. Int J Cancer, 2008. 122(10): p. 2286–93. [PubMed: 18224687]
78. Maruyama T, et al. , CCL17 and CCL22 chemokines within tumor microenvironment are related to infiltration of regulatory T cells in esophageal squamous cell carcinoma. Dis Esophagus, 2010. 23(5): p. 422–9. [PubMed: 20002703]

79. Daigo K, Mantovani A, and Bottazzi B, The yin-yang of long pentraxin PTX3 in inflammation and immunity. (1879–0542 (Electronic)).
80. Rusnati M, et al., Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. (0006–4971 (Print)).
81. Rodriguez-Grande B Fau - Varghese L, et al., Pentraxin 3 mediates neurogenesis and angiogenesis after cerebral ischaemia. (1742–2094 (Electronic)).
82. Rajkovic I, et al., Pentraxin 3 promotes long-term cerebral blood flow recovery, angiogenesis, and neuronal survival after stroke. (1432–1440 (Electronic)).
83. Bauvois B, New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. Biochim Biophys Acta, 2012. 1825(1): p. 29–36. [PubMed: 22020293]
84. Brooks PC, et al., Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. (0092–8674 (Print)).
85. Chetty C, et al., MMP-2 alters VEGF expression via alphaVbeta3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. (1097–0215 (Electronic)).
86. Dave JM, et al. , Hic-5 mediates the initiation of endothelial sprouting by regulating a key surface metalloproteinase. J Cell Sci, 2016. 129(4): p. 743–56. [PubMed: 26769900]
87. Chodankar R, et al. , Hic-5 is a transcription coregulator that acts before and/or after glucocorticoid receptor genome occupancy in a gene-selective manner. Proc Natl Acad Sci U S A, 2014. 111(11): p. 4007–12. [PubMed: 24591583]
88. Fujimoto N, et al. , Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate. J Biol Chem, 1999. 274(12): p. 8316–21. [PubMed: 10075738]
89. Shola DT, et al. , Hic-5 controls BMP4 responses in prostate cancer cells through interacting with Smads 1, 5 and 8. Oncogene, 2012. 31(19): p. 2480–90. [PubMed: 21996749]
90. Shibanuma M, et al. , A LIM protein, Hic-5, functions as a potential coactivator for Sp1. J Cell Biochem, 2004. 91(3): p. 633–45. [PubMed: 14755691]
91. Foster CT, Gualdrini F, and Treisman R, Mutual dependence of the MRTF-SRF and YAP-TEAD pathways in cancer-associated fibroblasts is indirect and mediated by cytoskeletal dynamics. Genes Dev, 2017. 31(23–24): p. 2361–2375. [PubMed: 29317486]
92. Du X, et al. , HIC-5 in cancer-associated fibroblasts contributes to esophageal squamous cell carcinoma progression. Cell Death Dis, 2019. 10(12): p. 873. [PubMed: 31740661]
93. Latini FR, et al. , Re-expression of ABI3-binding protein suppresses thyroid tumor growth by promoting senescence and inhibiting invasion. Endocr Relat Cancer, 2008. 15(3): p. 787–99. [PubMed: 18559958]
94. Zhang F, et al. , Genome-wide copy number variation study and gene expression analysis identify ABI3BP as a susceptibility gene for Kashin-Beck disease. Hum Genet, 2014. 133(6): p. 793–9. [PubMed: 24442417]
95. Gu J, et al. , Overexpression of ADAMTS5 can regulate the migration and invasion of non-small cell lung cancer. Tumour Biol, 2016. 37(7): p. 8681–9. [PubMed: 26738863]
96. Nakada M, et al. , Human glioblastomas overexpress ADAMTS-5 that degrades brevican. Acta Neuropathol, 2005. 110(3): p. 239–46. [PubMed: 16133547]
97. Porter S, et al. , Dysregulated expression of adamalysin-thrombospondin genes in human breast carcinoma. Clin Cancer Res, 2004. 10(7): p. 2429–40. [PubMed: 15073121]
98. Nanno Y, et al. , Serum Elastase 1 Level as a Risk Factor for Postoperative Recurrence in Patients with Well-Differentiated Pancreatic Neuroendocrine Neoplasms. Ann Surg Oncol, 2018. 25(11): p. 3358–3364. [PubMed: 30054822]
99. Liu J, et al. , CLEC3B is downregulated and inhibits proliferation in clear cell renal cell carcinoma. Oncol Rep, 2018. 40(4): p. 2023–2035. [PubMed: 30066941]
100. Lu X, et al. , Down-regulation of CLEC3B facilitates epithelial-mesenchymal transition, migration and invasion of lung adenocarcinoma cells. Tissue Cell, 2022. 76: p. 101802. [PubMed: 35500520]
101. Jones VA, et al. , The Role of Collagen XVII in Cancer: Squamous Cell Carcinoma and Beyond. Front Oncol, 2020. 10: p. 352. [PubMed: 32266137]

102. Lourenço GJ, et al. , A high risk of occurrence of sporadic breast cancer in individuals with the 104NN polymorphism of the COL18A1 gene. Breast Cancer Res Treat, 2006. 100(3): p. 335–8. [PubMed: 16807676]
103. Li S, et al. , Knobloch Syndrome Associated with Novel COL18A1 Variants in Chinese Population. Genes (Basel), 2021. 12(10).
104. Wang KS, Liu XF, and Aragam N, A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder. Schizophr Res, 2010. 124(1–3): p. 192–9. [PubMed: 20889312]
105. Tan Y, et al. , High expression of COL5A2, a member of COL5 family, indicates the poor survival and facilitates cell migration in gastric cancer. Biosci Rep, 2021. 41(4).
106. Zeng XT, et al. , The clinical significance of COL5A2 in patients with bladder cancer: A retrospective analysis of bladder cancer gene expression data. Medicine (Baltimore), 2018. 97(10): p. e0091. [PubMed: 29517678]
107. Nash BM, et al. , Heterozygous COL9A3 variants cause severe peripheral vitreoretinal degeneration and retinal detachment. Eur J Hum Genet, 2021. 29(5): p. 881–886. [PubMed: 33633367]
108. Rad A, et al. , Identification of three novel homozygous variants in COL9A3 causing autosomal recessive Stickler syndrome. Orphanet J Rare Dis, 2022. 17(1): p. 97. [PubMed: 35241111]
109. Tang J, et al. , RSRC1 and CPZ gene polymorphisms with neuroblastoma susceptibility in Chinese children. Gene, 2018. 662: p. 83–87. [PubMed: 29653227]
110. Pourhanifeh MH, et al. , The role of fibromodulin in cancer pathogenesis: implications for diagnosis and therapy. Cancer Cell Int, 2019. 19: p. 157. [PubMed: 31198406]
111. Mayfosh AJ, Nguyen TK, and Hulett MD, The Heparanase Regulatory Network in Health and Disease. Int J Mol Sci, 2021. 22(20).
112. Shanmugalingam T, et al. , Is there a role for IGF-1 in the development of second primary cancers? Cancer medicine, 2016. 5(11): p. 3353–3367. [PubMed: 27734632]
113. Yeung TL, et al. , Anticancer Immunotherapy by MFAP5 Blockade Inhibits Fibrosis and Enhances Chemosensitivity in Ovarian and Pancreatic Cancer. Clin Cancer Res, 2019. 25(21): p. 6417–6428. [PubMed: 31332047]
114. Zhao L, et al. , Loss of microfibril-associated protein 5 (MFAP5) expression in colon cancer stroma. Virchows Arch, 2020. 476(3): p. 383–390. [PubMed: 31422503]
115. Mondal S, et al. , Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. Eur J Med Chem, 2020. 194: p. 112260. [PubMed: 32224379]
116. Kiziltan R, et al. , Nidogen-2: A new biomarker in colon cancer patients. Ann Ital Chir, 2022. 92: p. 88–92. [PubMed: 34593672]
117. Srisuttee R, et al. , Evaluation of NID2 promoter methylation for screening of Oral squamous cell carcinoma. BMC Cancer, 2020. 20(1): p. 218. [PubMed: 32171289]
118. Wang J, et al. , Silencing NID2 by DNA Hypermethylation Promotes Lung Cancer. Pathol Oncol Res, 2020. 26(2): p. 801–811. [PubMed: 30826972]
119. Yu ZH, et al. , NID2 can serve as a potential prognosis prediction biomarker and promotes the invasion and migration of gastric cancer. Pathol Res Pract, 2019. 215(10): p. 152553. [PubMed: 31362888]
120. Acharya M, et al. , Evaluation of the OPTC gene in primary open angle glaucoma: functional significance of a silent change. BMC Mol Biol, 2007. 8: p. 21. [PubMed: 17359525]
121. Wang P, et al. , An evaluation of OPTC and EPYC as candidate genes for high myopia. Mol Vis, 2009. 15: p. 2045–9. [PubMed: 19844586]
122. Pillai VS, et al. , Identification of prolargin expression in articular cartilage and its significance in rheumatoid arthritis pathology. Int J Biol Macromol, 2018. 110: p. 558–566. [PubMed: 29402456]
123. Güzel Ö, et al. , The Role of Pentraxin-3, Fetuin-A and Sirtuin-7 in the Diagnosis of Prostate Cancer. Urol J, 2021. 19(3): p. 196–201. [PubMed: 34655076]

124. Karamfilova V, et al. , Increased Serum Pentraxin 3 Is Associated with Prediabetes and Type 2 Diabetes in Obese Patients with Nonalcoholic Fatty Liver Disease. Metab Syndr Relat Disord, 2022. 20(2): p. 132–136. [PubMed: 34818080]

125. Wang G, et al. , Pentraxin-3 as a predictive marker of mortality in sepsis: an updated systematic review and meta-analysis. Crit Care, 2022. 26(1): p. 167. [PubMed: 35676730]

126. Aboulouard S, et al. , In-depth proteomics analysis of sentinel lymph nodes from individuals with endometrial cancer. Cell Rep Med, 2021. 2(6): p. 100318. [PubMed: 34195683]

127. Liang CW, et al. , Loss of SFRP1 expression is a key progression event in gastrointestinal stromal tumor pathogenesis. Hum Pathol, 2021. 107: p. 69–79. [PubMed: 33186588]

128. Mirzaeyan F, et al. , Concurrent Evaluation of the Expression and Methylation of secreted frizzled-related protein 2 along with beta-catenin Expression in Patients with non-M3 Acute Myeloid Leukemia. Iran J Med Sci, 2021. 46(3): p. 180–188. [PubMed: 34083850]

129. Jung IH, et al. , SVEP1 is a human coronary artery disease locus that promotes atherosclerosis. Sci Transl Med, 2021. 13(586).

130. Young TL, et al. , SVEP1 as a Genetic Modifier of TEK-Related Primary Congenital Glaucoma. Invest Ophthalmol Vis Sci, 2020. 61(12): p. 6.

131. Orend G and Chiquet-Ehrismann R, Tenascin-C induced signaling in cancer. Cancer Lett, 2006. 244(2): p. 143–63. [PubMed: 16632194]

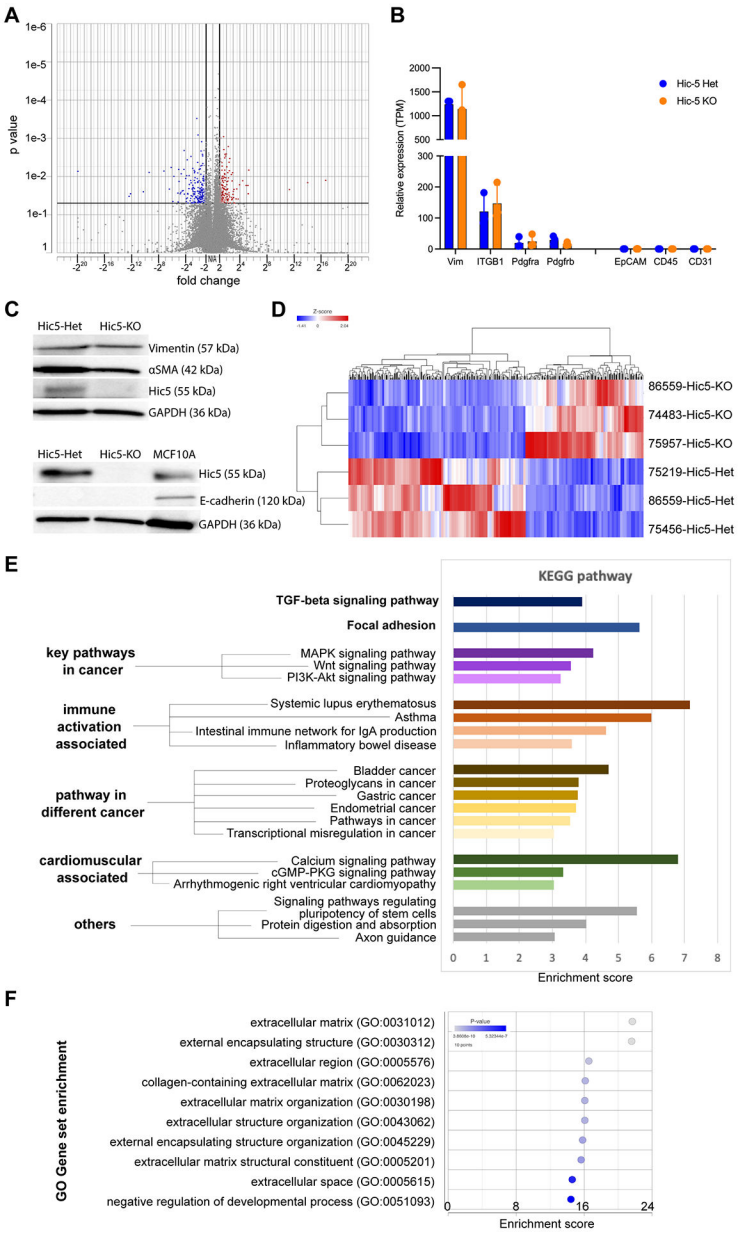

Figure 1.

RNA-seq analysis shows differentially expressed extracellular matrix (ECM)-associated genes in Hic-5 Het versus KO CAFs. (A) Volcano plot of the up-regulated (64) and down-regulated (138) genes in the Hic-5 KO CAFs (p-value <= 0.05, fold change < −2.5 or >2.5). (B) Expression levels (TPM) of fibroblast, epithelial and endothelial genes from the RNAseq of Hic-5 Het and Hic-5 KO CAFs. (C) Western blot of Hic-5 Het, Hic-5 KO CAFs and MCF10A epithelial cells. (D) Heat map of significantly changed genes in Hic-5 Het and KO CAFs. N=3 CAF isolates from mice per genotype. (E) Pathway enrichment analysis shows enrichment of key pathways identified by KEGG. (F) Gene set enrichment analysis shows enrichment of extracellular matrix-associated genes regulated by Hic-5 expression.

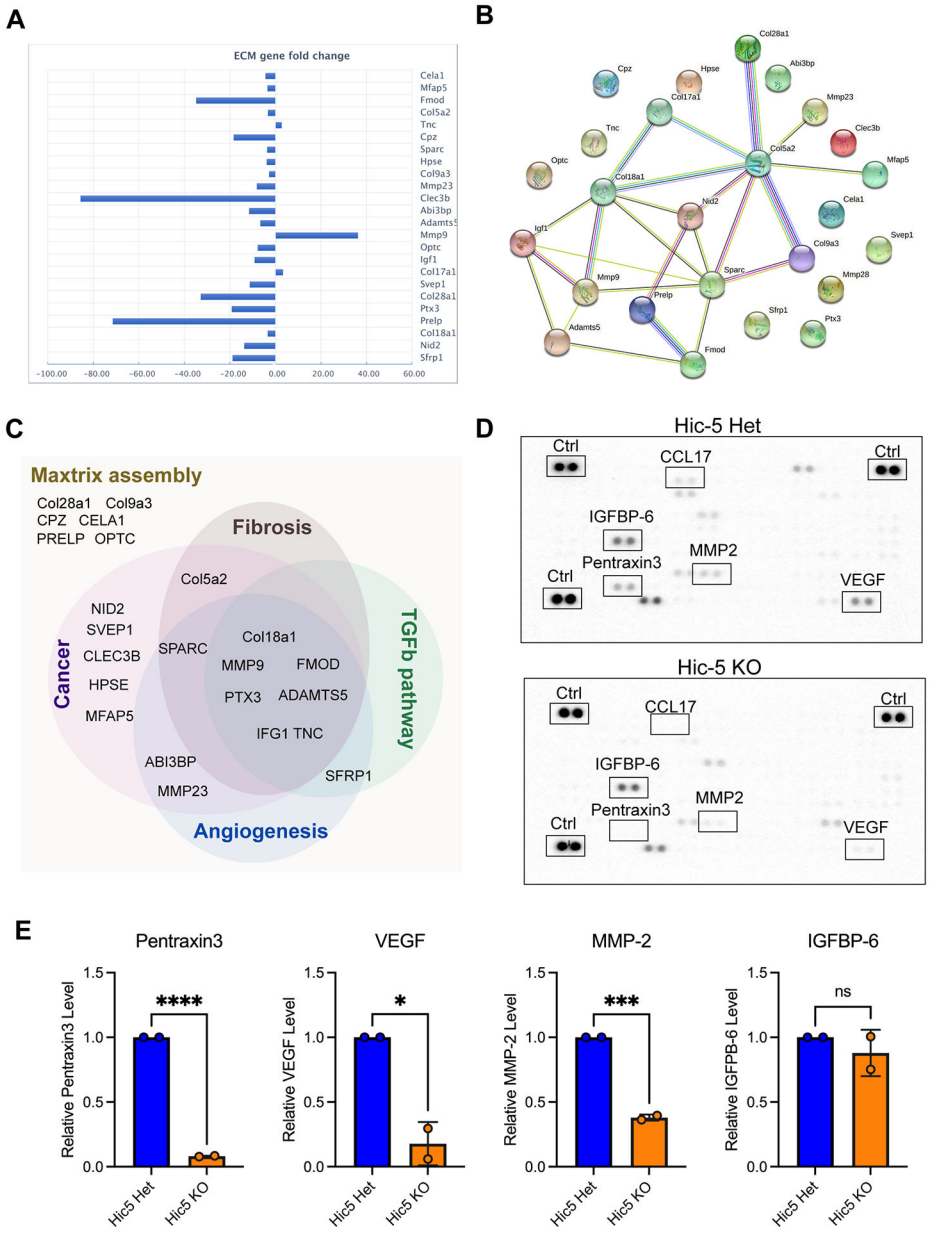

Figure 2.

Hic-5-regulated ECM genes are associated with cancer, fibrosis, TGF-β signaling and angiogenesis. (A) Relative expression, as determined by RNAseq analysis, of the 24 genes of the ECM signature, in Hic-5 KO versus Hic-5 Het CAFs. (B) Bioinformatics analysis by STRING to reveal functional interactions between the differentially expressed ECM genes. Cyan: interactions from curated database; purple: experimentally determined interactions; green: gene neighborhood; red: gene fusion; blue: gene co-occurrence; yellow: textmining; black: co-expression; light blue: protein homology. (C) Venn diagram highlighting the ECM genes interrelationship with key cancer associated pathways. (D) Representative cytokine array analysis from condition media of cultured Hic-5 Het and KO CAFs. (E) Quantification of angiogenesis-associated cytokine production from the cytokine array. Each dot represents

the average of duplicate values from two independent experiments n=2. A t-test was performed. Data are mean ± SD, *p<0.05, ***p<0.005, and ****p<0.001

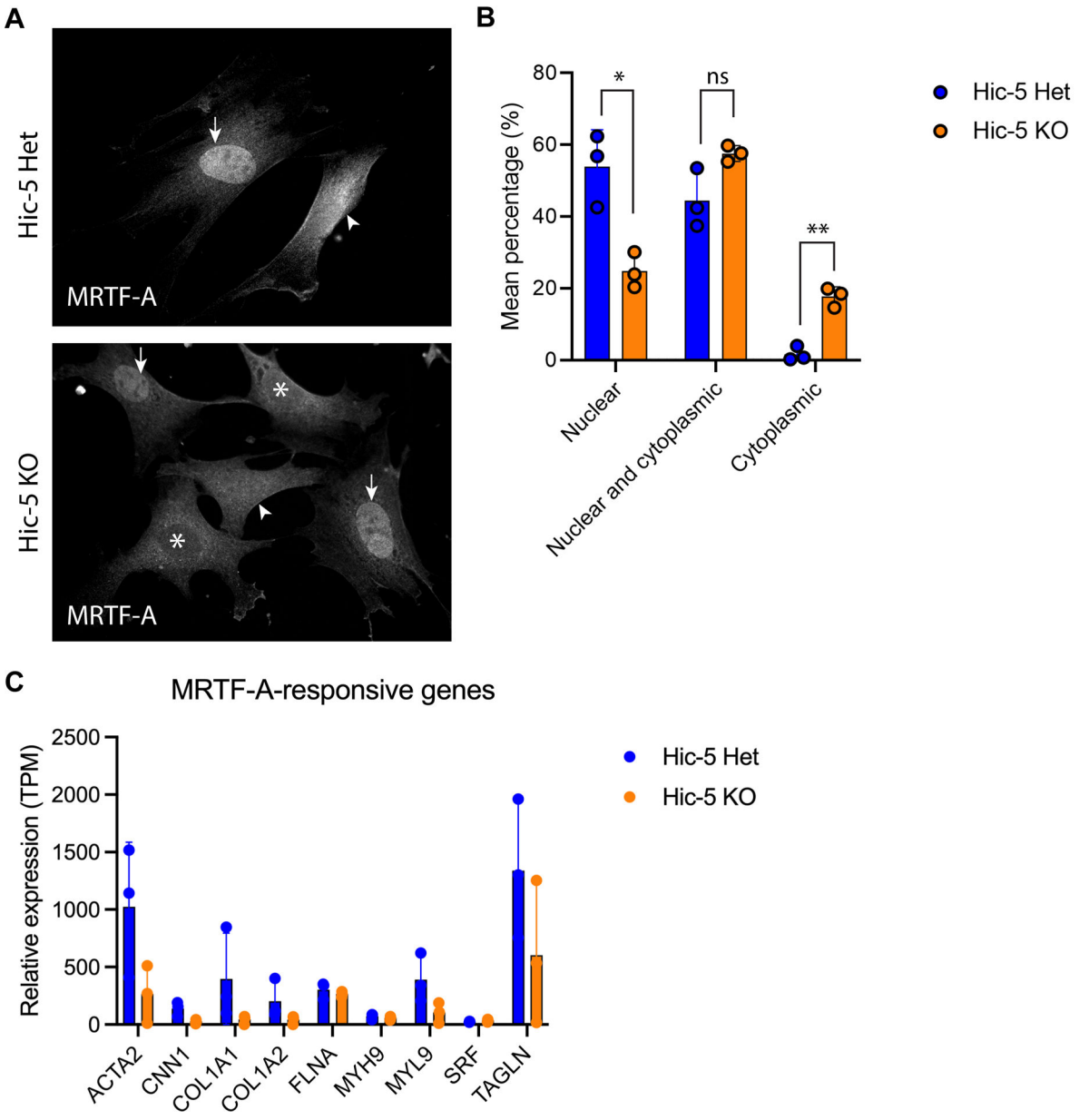

Figure 3.

Hic-5 expression regulates MRTF-A subcellular distribution. (A) Representative MRTF-A localization in Hic-5 Het and Hic-5 KO CAFs. Nuclear MRTF-A localization (arrows), nuclear and cytoplasmic MRTF-A (arrowheads), cytoplasmic MRTF-A (asterisk). (B) Quantification of the percentage of cells exhibiting the three MRTF-A localization phenotypes. N=3. Data presented as mean ± s.e.m. *p<0.05 **p<0.01 (C) Expression levels (TPM) of MRTF-A-responsive genes from the RNAseq of Hic-5 Het and Hic-5 KO CAFs.

Table 1.

Differential Extracellular Matrix genes

Gene Name	Description	Cancer / Disease	References
Abi3bp	ABI Family Member 3 Binding Protein	thyroid tumorigenesis; Kashin-Beck disease	[93, 94]
Adamts5	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 5	glioblastoma; NSCLC; breast cancer	[95–97]
Cela1	Chymotrypsin Like Elastase 1	pancreatic cancer	[98]
Clec3b	C-Type Lectin Domain Family 3 Member B	lung adenocarcinoma; clear cell renal cell carcinoma	[99, 100]
Col17a1	Collagen Type XVII Alpha 1 Chain	SCC; melanoma; breast cancer, cervical cancer; pancreatic carcinoma, thyroid cancer; colorectal cancer; lung cancer; salivary gland cancer	reviewed in [101]
Col18a1	Collagen Type XVIII Alpha 1 Chain	Knobloch Syndrome; breast cancer	[102, 103]
Col28a1	Collagen Type XXVIII Alpha 1 Chain	colon cancer; bipolar disorder	[104]
Col5a2	Collagen Type V Alpha 2 Chain	gastric cancer; prostate cancer; bladder cancer; colorectal cancer	[39, 105, 106]
Col9a3	Collagen Type IX Alpha 3 Chain	Stickler syndrome; retinal detachment;	[107, 108]
Cpz	Metallocarboxypeptidase Z	Neuroblastoma	[109]
Fmod	Keratan Sulfate Proteoglycan Fibromodulin	Chronic lymphocytic leukemia; glioblastoma; prostate cancer; colon cancer	reviewed in [110]
Hpse	Heparanase	atherosclerosis; fibrosis; breast cancer	reviewed in [111]
Igf1	Insulin Like Growth Factor 1	breast cancer; lung cancer; prostate cancer; colorectal cancer	reviewed in [112]
Mfap5	Microfibril Associated Protein 5	colon cancer; ovarian cancer; pancreatic cancer	[113, 114]
Mmp23	Matrix Metallopeptidase 23	melanoma	[61]
Mmp9	Matrix Metallopeptidase 9	breast cancer; cervical cancer	reviewed in [63, 115]
Nid2	Nidogen 2	colon cancer; oral squamous cell carcinoma; gastric cancer; lung cancer	[116–119]
Optc	Oculoglycan	high myopia; open-angle glaucoma	[120, 121]
Prelp	Proline And Arginine Rich End Leucine Rich Repeat Protein	rheumatoid arthritis; chronic leukemia	[56, 122]
Ptx3	Pentraxin 3	sepsis; prostate cancer; endometrial cancer; nonalcoholic fatty liver disease	[123–126]
Sfrp1	Secreted Frizzled Related Protein 1	acute myeloid leukemia; gastrointestinal stromal tumor	[127, 128]
Sparc	Secreted Protein Acidic And Cysteine Rich	bladder carcinoma; osteosarcoma; breast carcinoma; colorectal cancer; HNSCC; LSCC; gastric cancer; melanoma	[40–43, 45–50]
Svep1	Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	atherosclerosis; Congenital Glaucoma	[129, 130]
Tnc	Tenascin C	brain tumors; breast cancer; gynecologic cancer; prostate cancer; lung cancer; gastrointestinal cancer	reviewed in [131]