

Daily genetic profiling indicates JAK/STAT signaling promotes early hepatic stellate cell transdifferentiation

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Abstract

AIM: To identify signaling pathways and genes that initiate and commit hepatic stellate cells (HSCs) to transdifferentiation.

METHODS: Primary HSCs were isolated from male Sprague-Dawley rats and cultured on plastic for 0-10 d. Gene expression was assessed daily (quiescent to day 10 culture-activation) by real time polymerase chain reaction and data clustered using AMADA software. The significance of JAK/STAT signaling to HSC transdifferentiation was determined by treating cells with a JAK2 inhibitor.

RESULTS: Genetic cluster analyses, based on expression of these 21 genes, showed similar expression profiles on days 1-3, days 5 and 6, and days 7-10, while freshly isolated cells (day 0) and day 4 cells were genotypically distinct from any of the other days. Additionally, gene expression clustering revealed strong

upregulation of interleukin-6, JAK2 and STAT3 mRNA in the early stages of activation. Inhibition of the JAK/STAT signaling pathway impeded the morphological transdifferentiation of HSCs which correlated with decreased mRNA expression of several profibrotic genes including collagens, α -SMA, PDGFR and TGF β R.

CONCLUSION: These data demonstrate unique clustered genetic profiles during the daily progression of HSC transdifferentiation and that JAK/STAT signaling may be critical in the early stages of transdifferentiation.

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Key words: Cluster analysis; Fibrosis; Genetic profile; Hepatic stellate cell; Interleukin-6

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INTRODUCTION

Hepatic stellate cells (HSCs) play an important role in the development of liver fibrosis. Following exposure to a fibrogenic stimulus (e.g. virus, toxins, alcohol), the quiescent HSC transdifferentiated into an activated myofibroblast-like cell. During this process the HSC undergoes morphological changes (i.e. stellate cell to a stretched-polygon morphology), becomes hypercontractile and increases expression of fibrillar collagens and cytokines^[1]. Increased collagen deposition leads to accumulation of scar matrix,

the major cause of liver dysfunction during hepatic fibrosis^[2]. The transdifferentiation process, while very difficult to monitor *in vivo*^[3], can be seen and studied *in vitro*. HSCs *in vivo* undergo transdifferentiation when exposed to an altered microenvironment (e.g. increased type I collagen deposition as seen in fibrosis). This process can be mimicked *in vitro* by culturing these cells on a plastic substrate. Several groups have performed microarray analyses on both *in vitro* and *in vivo* HSC activation^[4,5]; however, little is known about the daily genetic alterations that occur. To understand this complex process, it is necessary to know the sequential activation of key genes, as well as the rise and fall of expression levels. Therefore, based on known gene expression profiles of the quiescent and activated HSC, several genes were selected to follow the transdifferentiation process throughout.

HSCs are an important source of cytokines, and cytokine cross-talk is the main pattern of cellular communication in the injured liver. Specifically, continual wound healing perpetuated by HSC transdifferentiation is associated with increased interleukin-6 (IL-6) expression, an important cytokine involved in the acute phase response observed post liver injury^[1]. IL-6 initially binds to specific receptor IL-6R (gp80) and subsequently two molecules of gp130 are recruited leading to activation of down-stream signaling. Classically, for induction of pro-inflammatory target genes, canonical JAK/STAT signaling is activated leading to increased inflammation as well as degradation of ECM^[6]. Signaling pathways such as the MAP kinase (MAPK) pathway are also transduced with the activation of soluble IL-6R^[7]. However, studies have shown that JAK/STAT signaling is the primary pathway for up-regulation of pro-inflammatory mediators/genes during acute phase response II, the body's innate immune response provoked as a result of liver injury^[7]. JAK/STAT downstream signaling affects expression of numerous genes including those involved in cellular proliferation and migration. Additionally, JAK/STAT signaling is associated with down-regulation of anti-apoptotic genes, including BCL-2 family proteins^[8]. Stimulation of proliferative pathways (MAPK) and increased cellular differentiation by JAK/STAT signaling promotes the fibrotic response and leads to increased activation of HSCs^[2]. Additionally, our lab has shown (unpublished data; Schrum lab) that JAK/STAT signaling increases collagen expression at both mRNA and protein levels supporting that this pathway is critical in modulating fibrosis.

To determine the daily genetic profile during normal transdifferentiation in HSCs, the expression of a mini-array of 21 genes (including members of the IL-6 JAK/STAT signaling pathway) across 10 d in culture was examined. Our results clearly demonstrate unique genetic profiles during different days of transdifferentiation and select days of activation showed similar patterns of gene expression. Results of the genetic and day cluster analyses suggest responsiveness of the cell to different signals will depend upon the temporal state of transdifferentiation. Inhibition of JAK/STAT signaling impeded the progression of HSC transdifferentiation as assessed morphologically and by gene expression. Thus, our data indicate that

JAK/STAT signaling may play a key role in the initiation of HSC transdifferentiation and that the changes in gene expression during a precise time period within the activation phase may determine the response of the HSC during this process.

MATERIALS AND METHODS

HSC isolation and culture

Primary HSCs were isolated from male Sprague-Dawley retired breeder rats (> 600 g) (Charles River, Raleigh, NC, USA). *In situ* liver perfusion using a pronase (Roche Molecular Biochemicals; Chicago, IL)/type I collagenase (Sigma-Aldrich; St. Louis, MO, USA) digestion was performed followed by Optiprep (Axis-Shield; Oslo, Norway) density gradient centrifugation. Cells were recovered at approximately 95% purity based on autofluorescence and washed with Gey's Balanced Salt Solution (GBSS: 137 mmol/L NaCl, 2.7 mmol/L NaHCO₃, 5.0 mmol/L KCl, 1.5 mmol/L CaCl₂·2H₂O, 1.0 mmol/L MgCl₂·6H₂O, 0.7 mmol/L Na₂HPO₄, 0.2 mmol/L KH₂PO₄, 0.3 mmol/L MgSO₄·7H₂O, 5.5 mmol/L glucose, 25 mmol/L HEPES) and either used immediately (termed: freshly isolated, Q or quiescent) or cultured on plastic using Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA), 2 mmol/L L-glutamine (Gibco), 100 units penicillin/mL, 0.1 mg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) in 5% CO₂ humidified atmosphere at 37°C. Growth media was changed every 2 d unless otherwise noted. All animal procedures were performed under the guidelines set by the University of North Carolina at Charlotte Institutional Animal Care and Use Committee and are in accordance with those set by the National Institutes of Health.

Treatment of HSCs

HSCs were either harvested immediately (day Q or quiescent) or were grown in culture for the designated number of days (every 24 h from the time of plating is considered 1 d). Cells were treated continually with 100 µmol/L AG490, a JAK2 inhibitor (CalBiochem, San Diego, CA, USA). Media during treatments was changed every 24 h.

Isolation of RNA and quantitative polymerase chain reaction

Total RNA was isolated from HSCs using TRIzol reagent (Gibco-BRL, Gaithersburg, MD, USA). RNA isolated from Q through day 3 was then cleaned using RNEasy Clean-Up (Qiagen) following the manufacturer's recommendations. Total RNA was reverse-transcribed using Superscript II reverse transcriptase (Promega, Madison, WI, USA) following the manufacturer's recommendations. Quantitative polymerase chain reaction (qPCR) was run at 94°C for 15 s; 58°C for 25 s; 72°C for 20 s, and read for 5 s (Table 1). The reaction mixture consisted of 1 µL each of cDNA, forward and reverse primers at 5 nmol/L, 2 µL DEPC water, and 5 µL of SYBR Green Master Mix

Table 1 Primers used in this study

Gene	Forward	Reverse	Size (bp)	Read (°C)
β -actin	GAGCTATGAGCTGCCTGACG	GGATGTCAACGTCACACTTC	154	80
Collagen $\alpha 1(I)$	CACTGCAAGAACAGCGTAGC	ATGTCCATTCCGAATTCCTG	200	82
Collagen $\alpha 2(I)$	AAGGCATTGAGGACACAAC	TTACCAACAGGCCCAAGTTC	296	84
Cyclin D1	TGGAAAGAAAGTGCCTGTGTG	TGCAAATGGAATGCTTCTG	291	82
Desmin	TACACCTGCGAGATTGATGC	ACATCCAAGGCCATCTTCAC	209	81
Focal adhesion kinase	TTACCCAGGTCAGGCATCTC	GGAATCGCTCTTCCTTTCC	218	80
Glyceraldehyde 3 phosphate dehydrogenase	ATCCCGCTAACATACCCTGG	ACTGTGGTCATGAGCCCTTC	292	80
Glial acidic fibrillary protein	AGAAAACCGCATCACCATTTC	TTGGGCCTAGCAAACAAGAC	264	81
Hypoxanthine-guanine phosphoribosyltransferase	CGCCAGCTTCCTCCTCAG	CCAGCAGGTCAGCAAAGAAC	288	80
Interleukin 6	CCCACCAGGAACGAAAGTC	TTTTCTGACAGTGCATCATCG	240	76
Interleukin 6 receptor/gp80	CAGGTGCCTTGCCAGTATTCT	AACTGACTTTGAGCCAACGA	228	79
Janus Kinase 2	GGTGCCCTAGGATTTTCTGG	CGACCAGCACTGTAGCACAC	284	79
Matrix metalloproteinase 13	CATACGAGCATCCATCCCGAGAC	GCATCTACTTTGTCGCCAATTC	290	78
Platelet-derived growth factor receptor β	ATGCAGTGCAGACTGTGGTC	CCGTGGTCATTACACTTCAC	247	82
Peroxisome proliferator-activated receptor γ	CGAGGACATCCAAGACAACC	CCGTCTTCTTGATCACAATGC	161	81
Ras homolog gene family, member A	CGGAATGATGAGCACACAAG	GCACCCCGACTTTTCTTC	207	80
Smooth muscle α -actin	CATCAGGAACCTCGAGAAGC	TCGGATACTCAGGGTCAGG	247	81
Signal transducer and activator of transcription 3	TATCTTGCCCTTTGGAATG	GTGGGGATACCAGGATGTG	284	80
Transforming growth factor β	ATGACATGAACCGACCCTTC	TGGTTGTAGAGGCAAGGAC	283	82
Transforming growth factor β receptor 1	GCTCTGGGTGTTTGGAG	CCAGCTCCTCACCCCTACAG	288	80

(Qiagen). The primers listed in Table 1 were all designed for rat. cDNA concentration was used as a reference to normalize samples since the expression of housekeeping genes was modulated through days in culture. Data were reported as cross-point, the point at which the detectable level of SYBR green fluorescence was detected above the background. All experiments were performed a minimum of three times, as noted.

Microscopy

For microscopic images, cells were visualized with transmission light microscopy at $200\times$ magnification. A second exposure was taken under fluorescent light with a DAPI filter to image the fluorescent retinol esters within the HSC using an Olympus IX71 microscope (Olympus America, Inc.; Hamburg, Germany). The white light image was overlaid with the fluorescent image to produce the final image.

Statistical analysis

Data are presented as mean \pm SE. One way repeated measures ANOVA was used for determination of statistical significance between the control and treatment groups using SigmaStat version 2.0. A *P* value of less than 0.05 was considered significant. AMADA 2.0.7 software was used to perform cluster analyses using Spearman correlation and average linkage^[9].

RESULTS

Gene expression profile time-course

To generate a daily profile of gene expression, 21 genes were selected as representatives of cellular behavior exhibited by HSCs. They included standard housekeeping genes (β -actin, G3PDH, HPRT), markers of quiescence [GFAP, peroxisome proliferator activated receptor γ (PPAR γ)], markers of activation (SMA, Desmin), matrix remodeling

genes [col $\alpha 1(I)$, col $\alpha 2(I)$, MMP13], mitotic and migratory associated genes (CycD, FAK, RhoA, PDGFR), and profibrotic cytokines, including the IL-6 JAK/STAT signaling pathway (IL-6, IL6R, JAK2, SOCS3, STAT3, TGF β , TGF β R). Total RNA was harvested at day Q and days 1-10 at exactly 24 h intervals ($n = 4$) and converted to cDNA for quantitative PCR analysis. These data were graphed as raw mRNA expression based on cycle number and subsequently normalized to total cDNA concentration. In order to examine relative fluctuations in gene expression, all genes were normalized to day Q (Figure 1A).

Since wide variations existed in mRNA expression of G3PDH, HPRT and β -actin based on total cDNA content (Figure 1A), a housekeeping gene was not used for normalization. For example, when IL-6 was normalized to a housekeeping gene (G3PDH, β -actin or HPRT), large variations in gene expression were observed (Figure 1B). Therefore, total cDNA concentration was used for normalization to quantitate daily changes in gene expression.

Due to the extensive amount of data generated from the mini-array, AMADA software was used to detect significant relationships in gene expression patterns. Genes with similar expression patterns over 10 d of culture were clustered together (Figure 2A). Genes incorporated in the same bracket have a more comparable expression pattern than those outside that bracket. The degree of correlation decreases with the distance between bifurcations^[10]. Further examination identified that SMA and collagen $\alpha 1(I)$ and $\alpha 2(I)$ were similarly regulated, while FAK, TGF β R and desmin displayed a similar yet not identical regulation as they were positioned on adjacent brackets. Conversely, GFAP, IL-6, JAK2, MMP13 and SOCS3 had distinctly different expression patterns as they were separated by multiple bifurcations. Additionally, PPAR γ demonstrated the least amount of relatedness to any other gene. Interestingly, a majority of IL-6 signaling pathway constituents

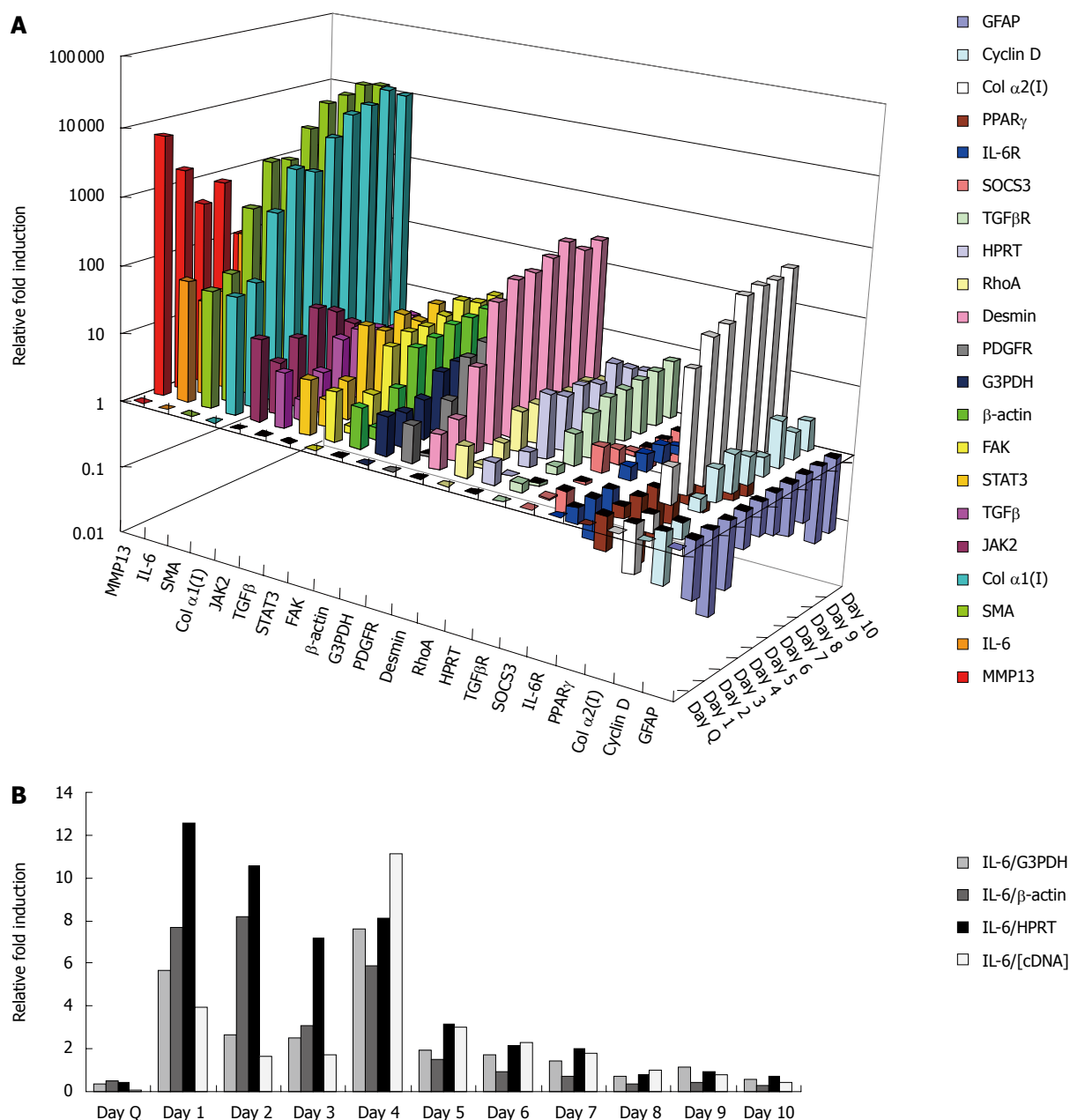


Figure 1 Gene expression profile time course. A: Mini-array of 21 genes across 10 d in culture as analyzed by real time polymerase chain reaction. Results are averages from three separate experiments. Quiescent time point was set to 1 for all genes; B: Classic housekeeping markers (G3PDH, β -actin and HPRT) fluctuated over days in culture activation, thus they served as unreliable normalization markers. IL-6: Interleukin 6; IL-6R: Interleukin 6 receptor; SOCS: Suppressors of cytokine signaling protein; TGF: Transforming growth factor; STAT: Signal transducers and activators of transcription; PPAR: Peroxisome proliferator activated receptor; FAK: Focal adhesion kinase; JAK: Janus kinase 2; SMA: Smooth muscle α -actin; MMP: Matrix metalloproteinase.

(IL-6, JAK2, SOCS3) were clustered together indicating a high degree of correlation in expression pattern, while STAT3 and IL-6R exhibited a divergent expression pattern from these three IL-6 signaling components.

Clustering software was utilized to demonstrate which days of culture-activation were most closely linked based on gene expression profile (Figure 2B). Day Q is distinct from all other days in culture. Days 1, 2 and 3 were closely linked, with day 1 having a more comparable gene expression profile to day 2 than day 3. Days 5 and 6 were also well-coupled as were days 7-10; however, as in all hierarchical clustering dendrograms, ordering within the bracket is arbitrary and thus does not contribute to the relatedness.

Day 4, like day Q, exhibited a distinct expression profile that did not directly correspond with other days in culture indicating that these days may be important cellular transition points.

Markers of gene expression

After examining gene expression by mini-array, we further dissected distinct markers of quiescence (GFAP, PPAR γ ; Figure 3A), markers of early activation (MMP13, IL-6; Figure 3B), markers of late activation (SMA, Desmin, col α 1(I), col α 2(I); Figure 3C), profibrotic markers (TGF β , TGF β R, MMP13, PDGFR; Figure 3D) and constituents of the IL-6 JAK/STAT signaling pathway (IL-6, IL6R,

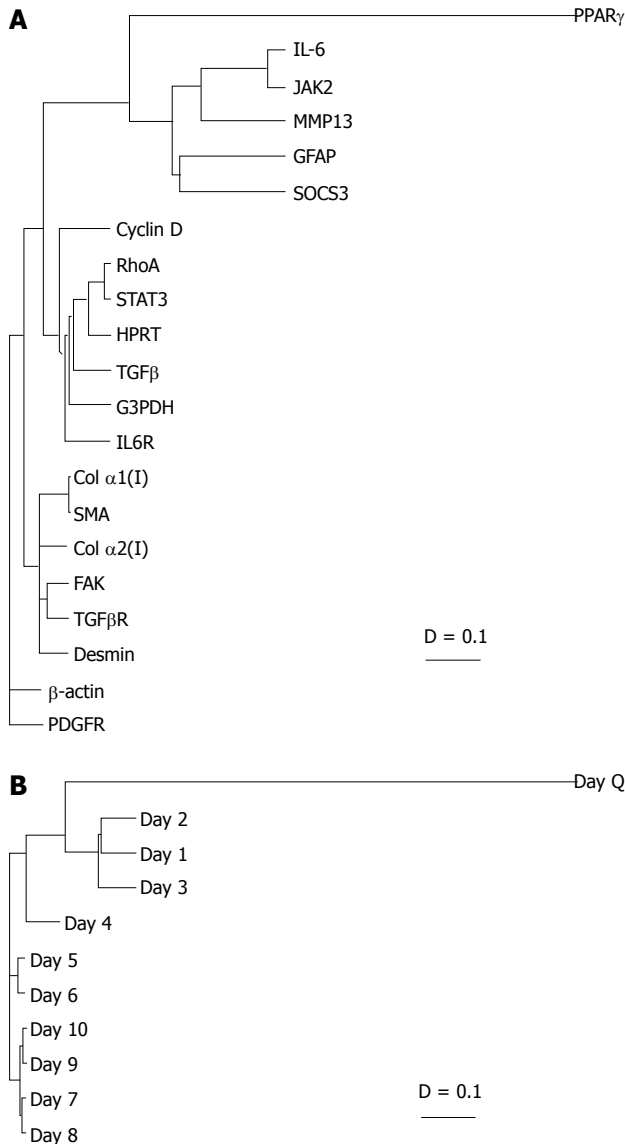


Figure 2 Clustered genetic and day analyses. A: The genes were grouped based on the degree of relatedness in their expression patterns over time using AMADA software. Distance relationships were calculated using Spearman rank correlation ($D = 0-2$). The shorter the distance between two genes, the more similar the expression profile over days in culture; B: The clustering tree demonstrated which days in culture were most coordinately regulated. As expected, day Q branches alone, while culture-activated HSCs are more closely regulated. Unexpectedly, day 4 also branched alone. IL-6: Interleukin 6; SOCS: Suppressors of cytokine signaling protein; TGF: Transforming growth factor; STAT: Signal transducers and activators of transcription; PPAR: Peroxisome proliferator activated receptor; FAK: Focal adhesion kinase; JAK: Janus kinase 2; SMA: Smooth muscle α -actin; MMP: Matrix metalloproteinase.

JAK2, SOCS3, STAT3; Figure 3E). Classic quiescent markers were clustered closely and decreased steadily over days in culture (Figure 3A), while markers of early activation increased sharply on day 1 and fell to basal levels over time (Figure 3B). Consistent with previous findings in our lab, we observed an approximate 10^3 -fold induction in MMP13 mRNA and a 100-fold induction in IL-6 mRNA expression from day Q to day 1. Markers of HSC activation increased steadily for the first seven days of activation then plateaued (Figure 3C). PDGFR, TGF β and TGF β R expression in-

creased after day 2 and remained constant (Figure 3D). Notably, five genes in the IL-6 JAK/STAT signaling pathway were coordinately regulated during early HSC transdifferentiation (Figure 3E). Although differences in gene expression magnitude existed, the overarching pattern remained consistent. These data suggest that the IL-6 JAK/STAT signaling pathway may be linked to regulation of HSC transdifferentiation which warrants further investigation.

JAK/STAT pathway in early HSC transdifferentiation

Since gene expression analysis indicated early transient spikes in IL-6, JAK2 and STAT3 mRNA (Figure 3E), the contribution of this signaling pathway to the initiation of HSC transdifferentiation was examined. A specific JAK2 inhibitor, AG490, was used to block JAK/STAT signaling within the cell to determine if inhibition of this pathway could alter changes seen in transdifferentiation. Cells were exposed continually to AG490 over a five day period and assessed morphologically at days 1, 3 and 5. Culture-activated HSCs lose retinol esters and cytoplasmic processes, and proliferate vigorously. As indicated by black arrows in Figure 4, cytoplasmic processes were only evident at day 1 in control cells compared to day 3 and 5 which exhibited stretched-polygon morphology characteristic of the activated phenotype. However, HSCs treated with AG490 retained these cytoplasmic processes over five days in culture indicating an inhibition of the activated phenotype and suggesting the importance of the JAK/STAT signaling pathway in early HSC transdifferentiation.

AMADA software was again used to cluster days based on gene expression profiles of the AG490-treated cells (Figure 5). Cluster analysis indicated that the expression profile of the HSCs treated with the inhibitor on days 1, 3 and 5 shared a high degree of relatedness to day Q as opposed to later days in culture. Specific gene expression associated with HSC activation was also examined. Inhibiting the JAK/STAT pathway with AG490 clearly showed a significant decrease in expression of markers of activation (collagen, SMA, PDGFR and TGF β R) thereby impeding early HSC activation (Figure 6). Day 3 showed the most prominent reduction in gene expression compared to day 1 and day 5. Even though significant decreases in gene expression were observed on day 5, a less dramatic effect was seen compared to day 3 suggesting that JAK/STAT may be involved in early transdifferentiation and plays a lesser role during later stages.

DISCUSSION

The process of HSC transdifferentiation is key in understanding and eventually ameliorating liver fibrosis. During the course of HSC transdifferentiation there are several genes/proteins that are altered in expression. Genes involved with lipid/vitamin A regulation^[11-13], such as PPAR γ ^[14,15], are lost, while cytoskeletal genes are shifted, decreasing glial fibrillary acidic protein (GFAP) but increasing desmin and smooth muscle α -actin (SMA)^[16-19]. Manipulation of PPAR γ expression by adenoviral expression

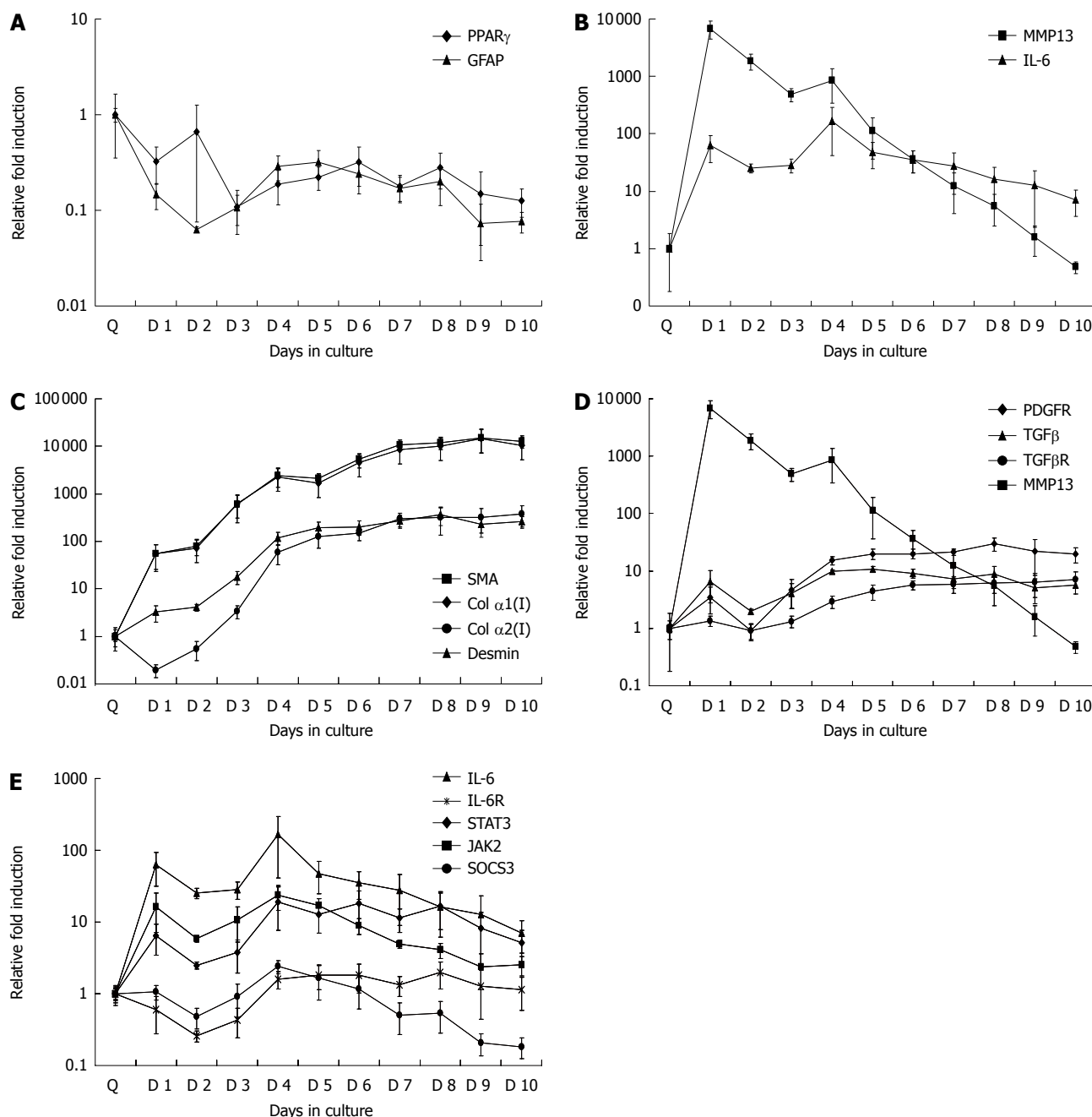


Figure 3 Gene expression markers during different stages of hepatic stellate cells activation. A: Markers of quiescence, early and late activation, fibrosis and the IL-6 signaling pathway (A-E, respectively) were examined over 10 d in culture. Real time polymerase chain reaction was used to analyze gene expression. Fold induction was relative to day Q expression levels. Data are presented as mean \pm SE. IL-6: Interleukin 6; IL-6R: Interleukin 6 receptor; SOCS: Suppressors of cytokine signaling protein; TGF: Transforming growth factor; STAT: Signal transducers and activators of transcription; PPAR: Peroxisome proliferator activated receptor; JAK: Janus kinase 2; SMA: Smooth muscle α -actin; MMP: Matrix metalloproteinase.

in activated cells leads to a reversal of the activated phenotype (flattened polygonal morphology with prominent actin stress fibers) to the quiescent phenotype (retracted cytoplasm and appearance of processes)^[20]. These phenotypic changes are also correlated with decreased expression of HSC activation marker genes such as collagen and TGF β ^[14]. Extracellular matrix components change from normal basement matrix components, such as type IV collagen, to a fibrotic matrix, including type I collagen^[21-23]. In all previous studies, quiescent HSCs or HSCs in early activation have been compared to activated cells. Although

continuous changes throughout the transdifferentiation process have been observed, changes in gene expression/profiles have not been tracked in a daily manner defining which days show similar phenotype and which days serve as a transition. While individual components of HSC phenotypes have been identified, there has been no concerted attempt to demonstrate the interplay of relevant genes as they coordinate the transition from a quiescent to a fully activated phenotype.

The initial stimulus of the transdifferentiation process has not been identified; however, several cytokines includ-

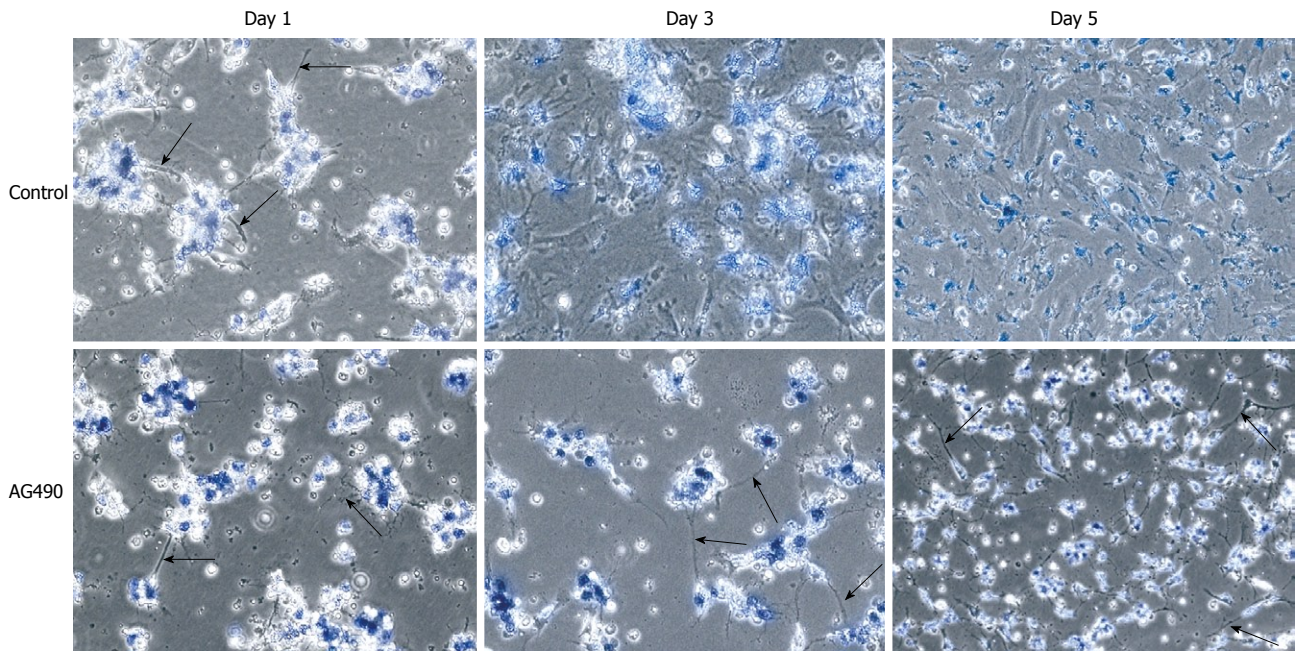


Figure 4 Inhibition of JAK/STAT signaling pathway: morphological alterations. Culture activated hepatic stellate cells were treated continually with AG490 throughout a five-day period and subsequently analyzed morphologically at days 1, 3 and 5. Cells treated with AG490 maintained their cytoplasmic projections (black arrows) throughout the five days of culture activation, whereas the control cells lost these processes as early as day 3.

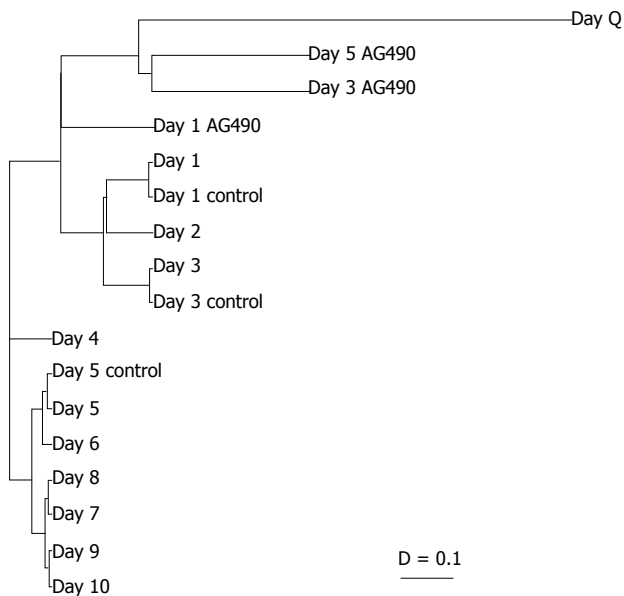


Figure 5 Inhibition of JAK/STAT signaling pathway: cluster analyses. AMADA software was used to detect any relationships between the days in culture treated with or without AG490 over a five-day period. Distance relationships were calculated using Spearman rank correlation ($D = 0-2$). The shorter the distance between two genes, the more similar the expression profile over days in culture. Data from the treated cells (days 1, 3 and 5) were incorporated with the previous data for days in culture (day Q-day 10) to demonstrate days of similarity based on gene expression. The clustering showed that the gene expression profile of HSCs treated with AG490 was highly related to the expression profile of the day Q hepatic stellate cells.

ing TGF β , TNF α and IL-6 have been implicated^[24]. Several labs have previously used microarray analysis to examine differential expression of genes in HSCs^[4,25]. However, these studies examined two or three specific time points,

rather than daily changes or gene relationships that could play a role in the initiation of transdifferentiation. In a study by De Minicis *et al.*^[4], HSCs cultured for 20 h represented the quiescent phenotype and day 5 culture-activated cells were considered fully activated. In another study, days 0, 4 and 7 were the selected populations^[25]. Furthermore, other studies examined cells at days 0 and 15 along with a third time point where cells were cultured until they had been passaged six or seven times^[26]. The aforementioned studies are not consistent with the days HSCs are considered to be quiescent (ranging from freshly isolated to day 1 or 2) or activated (ranging from days 5 to 15 of culture-activation). Varying degrees of quiescence and activation can lead to inconsistent results and misinterpreted data. None of these studies followed the transdifferentiation process on a daily basis. Therefore, these previous studies could not pinpoint transitions and steady-state profiles. Additionally, because of the multitude of time points, data generated in these studies are not comparable to each other. Therefore, for the first time, our study analyzed an array of 21 genes over consecutive days in culture to identify candidate signaling pathways and genes which peak in expression to initiate and commit the HSC to activation/transdifferentiation.

Using AMADA software, genotypic clustering was performed to profile genes that are modulated in quiescence, early and late activation, and profibrotic conditions. This analysis demonstrated that day Q was clearly divergent from all other days during transdifferentiation, while days 1-3 were significantly different from days 5-10 (Figure 2B). Furthermore, day 4 was genetically distinct from other days in culture, suggesting this may be the commitment point to the activated phenotype. Although no dramatic changes

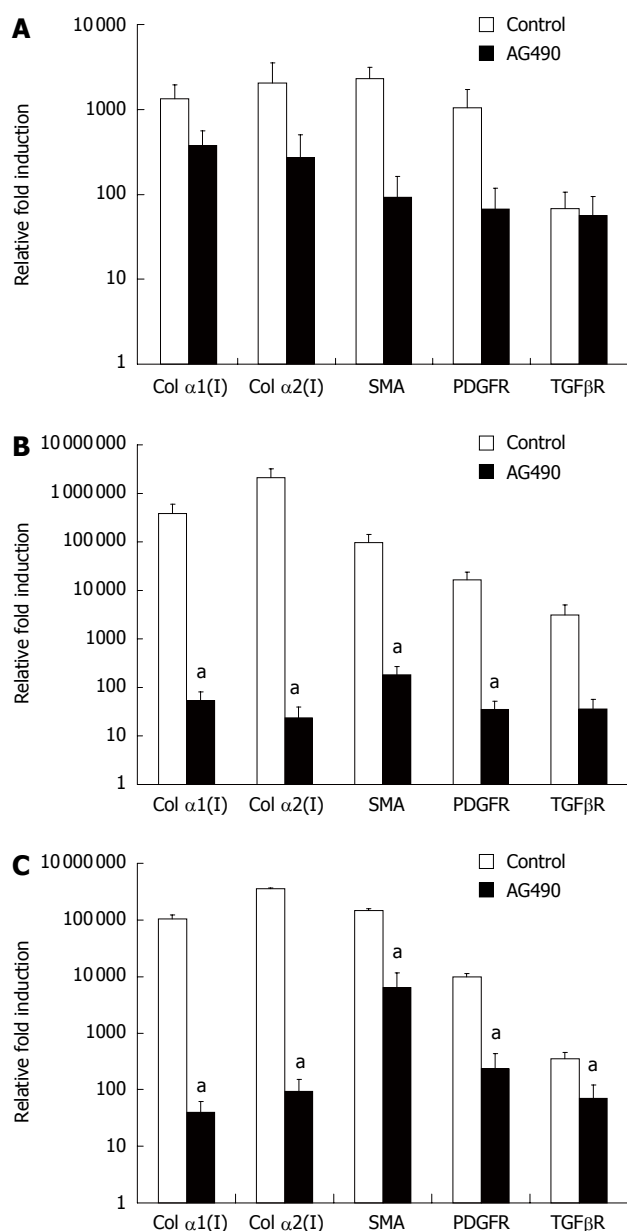


Figure 6 Inhibition of JAK/STAT signaling pathway: genetic alterations of fibrotic markers. mRNA expression of fibrotic genes from days 1, 3 and 5 (A-C, respectively) culture-activated hepatic stellate cells treated with or without AG490 were analyzed by real time polymerase chain reaction. Data were presented as mean \pm SE. * $P < 0.05$ compared to control.

in gene expression were seen at day 4, commitment to expression patterns was observed suggesting that day 4 is a critical point of transition (Figure 3). Additionally, components of the IL-6 JAK/STAT signaling pathway appeared to be crucial in the first 72 h of HSC transdifferentiation.

Our results also demonstrated that blocking the JAK/STAT signaling pathway inhibited phenotypic changes seen during activation as indicated by retention of cytoplasmic projections (Figure 4) and significantly decreased profibrotic gene expression (Figure 6). JAK/STAT signaling can be transduced by numerous factors/ligands including IL-6, leptin and interferon γ (IFN- γ) which are also associated with HSC activation^[24,27].

IL-6 is upregulated in liver disease, with positive effects

on liver regeneration and protection from hepatotoxins. IL-6 is an important cytokine in the regulation of immune and acute phase responses during bacterial infections or damage^[28]. It can be synthesized by a variety of liver cells, including Kupffer cells (KCs), hepatocytes^[29] and HSCs^[30-33], as well as infiltrating lymphocytes, monocytes/macrophages, endothelial cells, smooth muscle cells and fibroblasts^[28,34]. Conversely, IL-6 signaling can also be detrimental. In the case of liver fibrosis, it is profibrotic causing perpetual type I collagen stimulation by HSCs^[28]. Thus, fibrogenic effects of autocrine and paracrine IL-6 signaling and the specific contribution of each on initiation of HSC activation and perpetuation warrants further investigation.

The profibrotic hormone, leptin, binds to OB-R1 transducing its signal through the JAK/STAT complex. Culture-activated but not quiescent HSCs express leptin^[35] resulting in significant increases in α 2(I) collagen mRNA^[36] and suppression of PPAR γ ^[37]. Inhibition of JAK2 with AG490 impeded leptin signaling thereby ameliorating the fibrotic response^[38]. In contrast, IFN- γ is considered to be anti-fibrotic and also signals through the JAK/STAT pathway. The profibrotic cytokine, TGF β , increases ECM deposition from activated HSCs contributing to disruption of liver architecture in fibrosis. IFN- γ has been shown to abrogate the activation of TGF β and diminish the excessive wound healing response^[39]. While it is clear that JAK/STAT signaling is important in the fibrotic response of HSCs, the specific ligand initiating transdifferentiation is yet to be elucidated. Numerous studies have demonstrated that signaling through the MAP kinase (MAPK) pathway leads to increased HSC proliferation promoting the fibrotic response^[2,7]. However, JAK/STAT signaling is the primary pathway for up-regulation of pro-inflammatory mediators/genes during liver injury^[7], and no studies to date have examined the significance of JAK/STAT signaling during early HSC transdifferentiation.

We describe here that freshly isolated HSCs are distinct from all days of culture-activation and that these cells should not be equated with or used interchangeably with early days in culture. Based on our daily genetic profile assessment, the day of culture-activation used could significantly impact studies examining different cellular processes including transdifferentiation, proliferation, gene expression and migration of HSCs. It would be interesting to determine if other fibrogenic stimuli such as alcohol or acetaldehyde could also initiate HSC activation through JAK/STAT, particularly given that commitment to the activated phenotype perpetuates the fibrotic response. Overall, these data demonstrate that the daily changing genetic profile of the HSC results in differentiation into a unique phenotype rendering the cell sensitive to ligands which signal through JAK/STAT. Since inhibition of the JAK/STAT signaling pathway impedes the progression of HSC transdifferentiation/activation, this may serve as a potential therapeutic target to inhibit or slow the development of liver fibrosis.

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COMMENTS

Background

Chronic liver disease is among the top ten disease related causes of death in the US (CDC, National Center for Health Statistics, 2007). One of the primary forms of chronic liver disease is liver fibrosis, which is mediated by a number of factors including viral infection, genetic disease, and/or xenobiotic-induced damage. The hepatic stellate cell (HSC) plays a crucial role in the development and progression of liver disease to fibrosis.

Research frontiers

In the normal liver the HSC resides in a quiescent state; however, upon hepatic injury, the HSC transdifferentiated (activates) into a myofibroblast-like cell characterized by increased proliferation, extracellular matrix production and cell survival. However, the signaling molecules responsible for this activation process have not been fully elucidated. In this study the authors demonstrate that the JAK/STAT signaling pathway is critical for early HSC activation.

Innovations and breakthroughs

Numerous studies have shown that several profibrogenic genes are transduced by the JAK/STAT signaling pathway. This is the first study showing that JAK/STAT inhibition impedes initiation of HSC activation. Additionally, this study showed through a daily genetic profile assessment that the day of culture-activation used can significantly impact studies examining different cellular processes including transdifferentiation, proliferation, gene expression and migration of HSCs.

Applications

Understanding factors that regulate HSC activation is crucial for the development of therapeutic interventions for the treatment of liver fibrosis.

Terminology

JAK/STAT signaling communicates extracellular information to the cell's nucleus initiating target gene transcription influencing the fibrotic status. In order to signal through the JAK/STAT pathway, a cell membrane receptor, JAK and STAT are required. Transdifferentiation of the HSC is a transformation process in which the cell changes from one type to another. This process is critical for the development of liver fibrosis. Culture-activation refers to the routine practice of growing HSCs on plastic dishes in the lab to mimic the transdifferentiation process that occurs in the liver.

Peer review

This is a very well structured piece of basic research. It adds to the growing literature on HSC mechanisms leading to fibrogenesis. It is a well written manuscript, especially the discussion section is interesting. It includes comprehensive literature relevant to discussion.

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