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Application of microarrays to identify and characterize genes involved in attachment dependence in HeLa cells

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Abstract

The ability to modify cellular properties such as adhesion is of interest in the design and performance of biotechnology-related processes. The current study was undertaken in order to evaluate the effectiveness of modulating cellular adhesion in HeLa cells from a genomics perspective. Using DNA microarrays, differences in gene expression between two phenotypically distinct, anchorage-dependent and anchorage-independent, HeLa cell lines were identified. With the aid of several statistical methods and an extensive literature search, two genes were selected as potential targets for further study: *siat7e* and *lama4*.

Subsequently, experiments were carried out to investigate the effects of *siat7e* and *lama4* separately, on adhesion in HeLa cells by altering their expression *in vivo*. Decreasing the expression of *siat7e*, a type II membrane glycosylating sialyltransferase, in anchorage-independent HeLa cells using short interfering RNA (siRNA) resulted in greater aggregation (i.e. clumping) and morphological changes as compared to untreated anchorage-independent HeLa cells. Similar effects were seen in anchorage-independent HeLa cells when the expression of *lama4* which encodes laminin $\alpha 4$, a member of the laminin family of glycoproteins, was enhanced as compared to untreated anchorage-independent HeLa cells. Using a shear flow chamber, an attachment assay was developed; illustrating either increased expression of *siat7e* or decreased expression of *lama4* in anchorage-dependent HeLa cells reduced cellular adhesion.

Collectively, the results of this study are consistent with the roles *siat7e* and *lama4* play in adhesion processes *in vivo* and indicate modifying the expression of either gene can influence adhesion in HeLa cells. The strategy of applying bioinformatics techniques to characterize and manipulate phenotypic behaviors is a powerful tool for altering the properties of various cell lines for desired biotechnology objectives.

Keywords

HeLa; laminin; sialyltransferase; cellular adhesion; sialic acid; aggregation; genomics

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Introduction

In the design of biological processes for the production of therapeutic and/or diagnostic compounds from mammalian cells, it is vital to take into account the properties of the cell line being used (Chu et al., 2005). Details pertaining to a cell line in terms of its: growth pattern, nutritional requirements, glycosylation capabilities, and response to stimuli are important parameters to consider in order to properly design specific processes (Lum et al., 2004).

An important cellular property in biotechnology applications is adhesion—a cell's ability to attach to a surface in order to grow (Zhu et al., 2002). Depending on the exact application, a cell line that does not adhere to a surface, anchorage-independent, may be strongly preferred over a cell line that adheres to a surface, anchorage-dependent (Lum et al., 2004; Karu et al., 2001). For a different application, an anchorage-dependent cell line may be preferable over an anchorage-independent cell line. Being able to manipulate the cellular feature of adhesion would, therefore, benefit biotechnology applications and is the basis of the current study (Lum et al., 2004; Zhu et al., 2002).

A variety of studies have been conducted to evaluate the importance of cellular properties for the production of specific products. Researchers have also identified possible pathways to modify cellular properties by employing specific selection methods. In relation to adhesion, most studies have focused on either quantifying observations on a genetic level or exploring the effects of specific compounds (Springer et al., 1976). For instance, selenite, a hydrous calcium sulfate, has been shown to reduce the ability of HeLa cells to attach to fibronectin (Zhu et al., 1998). Researchers also showed that blocking the expression of *pten*, a tumor suppressor gene, in 293T cells using siRNA resulted in a loss of adhesion as well as a change in morphology (Mise-Omata et al, 2005; Crowther, 2002). Additional studies have highlighted a number of genes thought to be involved in mediating adhesion such as *rhoA*, *rac1*, and *cdc42*; although the exact mechanisms have not been fully elucidated (Mise-Omata et al, 2005; Hatzimanikatis and Lee, 1999; Tavazoie et al., 1999).

In the present study DNA microarrays were used to identify genomic differences between anchorage-independent and anchorage-dependent HeLa cells. Data generated from these arrays were normalized and then screened using a variety of imaging tools. Based on the results of several clustering algorithms, a review of previous studies, and their relative expression levels, two genes were selected for further investigation. The expression levels of these two genes, *siat7e* and *lama4*, were verified using reverse transcription-polymerase chain reaction (RT-PCR). Once verified, the expression level of either gene was manipulated and the adhesion features of these 'altered' cells were characterized first using a particle counter and then a shear flow chamber.

Materials & Methods

1. Cell Culture Growth & Sampling

The two cell lines, anchorage-dependent and anchorage-independent HeLa cells, were obtained from the American Type Culture Collection (ATCC, Manassas, VA) (Catalog Nos. CCL-2 and CCL-2.2, respectively). Both cell lines were grown in BioFlo 3000 bioreactors (New Brunswick Scientific Co., Edison, NJ) with a working volume of 1.5L. Runs were conducted for up to 7 days after inoculation with constant sampling to characterize growth parameters. At least two different runs were carried out for each cell line. The media used was DMEM (Biosource International, Camarillo, CA) with 10% FBS (Biosource International, Camarillo, CA). The anchorage-dependent HeLa cells were grown on Cytodex 3 (Amersham Biosciences, Piscataway, NJ) microcarriers. Each reactor was seeded with 5.0×10^5 cells/mL (Bleckwenn et al., 2005; Masters, 2002).

Samples from the bioreactors were taken at regular intervals to test media composition (i.e. pH, Glucose, Lactate), cell viability, and cell density. Cells used to seed the bioreactors were synchronized using serum deprivation for 24 hours (Simon et al., 2002). Samples were collected in 2mL RNase/DNase-free micro tubes (Marsh Biomedical Products, Rochester, NY), combined with TRIzol reagent (Invitrogen, Carlsbad, CA) and stored at -80°C .

2. RNA Isolation & Sample Preparation

Total RNA was isolated from samples using an Invitrogen kit (Micro-to-Midi Total RNA Purification System). Purified RNA was quantified using a spectrophotometer, GeneQuant Pro (Biochrom Ltd, Cambridge, UK). The absorbance values at 260nm and 280nm were measured and the quality of RNA was determined by the ratio of these numbers ($A_{260/280}$). Only samples with an $A_{260/280}$ of at least 1.8 were used for microarray analysis (Butte, 2002). Each sample, consisting of approximately 10 μg of total RNA, was reverse transcribed, labeled, and prepared for microarray hybridization using a protocol from The Institute for Genomic Research (TIGR) in Rockville, MD: "Aminoallyl labeling of RNA for Microarrays" (SOP # M004, Rev. 2) with an effective date of 3/4/2002.

3. DNA Microarray Preparation, Analysis, and Subsequent Verification

High quality microscope slides (Corning, Corning, NY) were printed with a set of 32,448 spots corresponding to approximately 14,000 unique ESTs (genes) by TIGR (Rockville, MD) using an array fabricator (Intelligent Automation, Rockville, MD). These complementary DNA (cDNA) microarrays were prepared and hybridized using a protocol "Microarray labeled probe hybridization" (SOP # M005, Rev. 3) available from TIGR with an effective date of 9/11/2002. After washing and drying, each slide was ready for scanning and image analysis using a GenePix 4000B (Molecular Devices Corporation, Sunnyvale, CA). To ensure proper labeling efficiency the dyes for 2 arrays were swapped (Lee and Quackenbush, 2000).

Acuity software (Molecular Devices Corporation, Sunnyvale, CA) was used to normalize, filter, and cluster the data. Due to similarities between the two cell lines, total intensity normalization was deemed most appropriate and therefore applied to the data (Xiang et al., 2003;Burke, 2000). Next, the data were filtered as part of a quality control step to remove spots with poor signal quality, defined as having low total signal, non-circular shapes, or a signal below local background (Saeed et al., 2003;Conway and Schoolnik, 2003; Hegde, et al., 2000). In addition, genes with highly variable (i.e. inconsistent) expression ratios were removed.

In order to explore the data further, the following clustering algorithms were applied: self-organizing maps (SOMs), principle component analysis (PCA), and hierarchical clustering (Saeed et al., 2003,Quackenbush, 2001). PCA was used to estimate the number of distinct clusters likely to form in the data (Quackenbush, 2001). This algorithm revealed 8 or 13 distinct clusters would best segregate the data, based on the inherent structure of the data. SOMs and hierarchical clustering were then applied in order to construct groups of genes with potentially similar functions or roles based on expression patterns. The parameters typically specified for either of these algorithms such as scaling, the similarity metric, or the linkage method were continually modified to best separate the data into meaningful groups. Once a final value for each of these parameters was selected, the resulting groups were probed to identify subsets of genes with relevance to adhesion. This was done by searching for genes closely associated with other genes known to be involved in adhesion such as integrins and cadherins. Of these genes, the most differentially expressed were then identified and evaluated based on their known or proposed functionalities as indicated in the literature. Using this approach, two genes were selected for further investigation; *siat7e* and *lama4*.

To enhance statistical significance associated with gene-specific expression levels, four oligonucleotide microarrays were also hybridized. The arrays used were Human Genome U133 Plus 2.0 (Affymetrix, Santa Clara, CA) prepared with over 54,000 spots corresponding to roughly 38,000 genes. Two of these arrays were hybridized with RNA extracted from anchorage-dependent HeLa cells while the remaining two arrays were hybridized with RNA extracted from anchorage-independent HeLa cells. The GeneChip arrays were assayed using the Affymetrix protocol “Eukaryotic sample and array processing” (701027, Rev. 5).

Although, the differences and similarities between cDNA and oligonucleotide microarrays are numerous, for the purposes of this text, only two differences are of particular interest; sample hybridization and resulting data. The cDNA microarrays utilized in the present study were dual-channel whereas the oligonucleotide microarrays were single-channel. When an array is hybridized with two samples, each labeled with a different dye, the array is referred to as being a dual-channel array. Conversely, when only one sample is hybridized onto an array, the array is referred to as being a single-channel array. As a result of this difference, data generated from the dual-channel arrays were in terms of relative intensity values (i.e. ratio form) whereas data from the single-channel arrays were in terms of absolute intensity values (Zhu et al., 2002; Nakanishi et al., 2001).

In addition, reverse transcription-polymerase chain reaction (RT-PCR) was performed to verify the microarray results. The protocols used were provided by Applied Biosystems (4333458, Rev. B & 4335626, Rev. C, both dated 05/2004). Each gene was assayed four times through two separate experiments to establish greater statistical significance.

4. Modifying gene expression levels of *siat7e* and *lama4* via transient transfections

Sequencing information for both *siat7e* and *lama4* were obtained using two public online databases; Harvester (<http://harvester.embl.de/>, European Molecular Biology Laboratory, Heidelberg, Germany) and GenBank (<http://www.ncbi.nlm.nih.gov/>, National Institutes of Health, Bethesda, MD). For each gene, full-length DNA constructs (GeneCopoeia, Germantown, MD) and siRNA were purchased (Ambion, Austin, TX). The siRNA sequences: siRNA ID # 11236 (*lama4*) and siRNA ID # 112233 (*siat7e*) were used.

For the anchorage-dependent HeLa cells, the transfecting agent used was Lipofectamine 2000 (Invitrogen, Carlsbad, CA), the transfecting agent used for the anchorage-independent HeLa cells was Lipofectamine LTX (Invitrogen, Carlsbad, CA). First, a frozen vial of the cell line to be transfected was thawed and then used to seed a 25cm² cell culture flask (Corning, Corning, NY). Within 24 hours, spent media was replaced with fresh media, DMEM (Biosource International, Camarillo, CA) with 10% FBS (Biosource International, Camarillo, CA). Upon reaching confluency, the cells were expanded; first into a 75cm² cell culture flask and then into a 162cm² cell culture flask. When the 162cm² flask was approximately 90% confluent, cells were passaged into two 6-well plates (Corning, Corning, NY) with fresh media. Once the cells were at least 80% confluent, transfection was performed. Samples for subsequent analysis were taken after 48 hours for verification of successful transfection using RT-PCR.

Physiological changes, post-transfection, were observed with a DM IRB microscope and attached camera (Leica Camera, Allendale, NJ). Quantification of observable changes was performed using a Counter Z (Beckman Coulter, Fullerton, CA). In order to assay the level of adhesion for a specific manipulation, the formation and distribution of cellular aggregates in both control and treated cells were analyzed. Two separate samples were prepared by diluting each in an electrolyte solution at a ratio of 1:2 and run through the Beckman Coulter counter for varying sizes. In addition, three different 6-well plates were seeded and analyzed.

To account for variability introduced during the transfection process the following controls were established: (i) untreated cells, (ii) cells treated only with the transfecting agent, Lipofectamine 2000 (Invitrogen, Carlsbad, CA), (iii) cells transfected with nonsense sequence siRNA, Silencer Negative siRNA Control # 1 (Ambion, Austin, TX), and (iv) cells transfected with Silencer GAPDH siRNA (Ambion, Austin, TX). Transfection with nonsense siRNA served as the negative control whereas transfection with siRNA specific for GAPDH served as the positive control (used to optimize conditions for transfection). Subsequently, the mRNA levels were monitored using RT-PCR. The data labeled 'control' in Figures 3 & 4 were calculated by averaging the values from all four controls previously mentioned, due to very similar distribution patterns.

Based on previous work with the anchorage-independent HeLa cells the following ranges (in μm) were selected for investigation: <5, 5-15, 16-30, 31-60, 61-80, 81-100, and >100 (Masters, 2002). These ranges were not meant to be definitive but rather suggestive of different possible groupings for HeLa cells with an average diameter of 12-15 μm . In addition, when taken together these ranges are free of gaps and therefore present a complete picture of the aggregation taking place. The first range (<5) represented debris and cellular fragments and therefore was considered background. The next two ranges (5-15 and 16-30) represented single cells, dividing cells, and pairs of cells. The 31-60 range represented small-to-medium sized aggregates of 3-6 cells. Finally, the last three ranges represented medium-to-large aggregates comprised of 6 or more cells.

5. Plasmid DNA inserts & Attachment Assay

The expression levels of either *siat7e* or *lama4* were altered by transfecting plasmid DNA conferring resistance to the compound geneticin (neomycin gene). This resistance allowed for selection of clones expressing the plasmid. To enhance the expression of either gene, a vector containing the full-length gene sequence was used. On the other hand, to reduce the expression of either gene, a vector containing the appropriate siRNA sequence was used. The stability of the cell lines with DNA plasmid inserts was tested using western blot analysis (*lama4*) and RT-PCR (*siat7e*). After every fourth passage, a western blot or RT-PCR was performed to verify continued repression or enhancement of the specific gene being targeted. Prior to transfection, a kill curve was constructed to optimize the concentration of geneticin incubated with the cells resulting in 100% cell death (750 $\mu\text{g}/\text{mL}$).

In order to distinguish these clones from one another and from the original cell lines, the following designations were used: anchorage-dependent [*lama4* -], anchorage-dependent [*siat7e* +], anchorage-independent [*lama4* +], and anchorage-independent [*siat7e* -]. The first two cell lines were prepared using anchorage-dependent HeLa cells. In the case of the anchorage-dependent [*lama4* -] cells, the expression of *lama4* was reduced by inserting a vector embedded with siRNA specific for *lama4*. Anchorage-dependent [*siat7e* +] cells were prepared by inserting an expression vector that contained both the full-length sequence of the gene *siat7e* and a CMV promoter (GeneCopoeia, Germantown, MD). Similarly, anchorage-independent [*lama4* +] cells were constructed from anchorage-independent HeLa cells by introducing an expression vector that contained the full-length *lama4* gene. The anchorage-independent [*siat7e* -] cells were also prepared from anchorage-independent HeLa cells using a vector that contained siRNA specific for *siat7e*.

To quantify adhesion properties in these modified cell lines, a shear flow chamber was utilized. The apparatus was placed onto the stage of an inverted microscope with a camera capable of generating real-time photos and video (Abulencia et al., 2003). By growing cells on a 35mm $\times$ 10mm cell culture dish (Corning, Corning, NY) cells could be placed directly inside the shear chamber. As such, varying levels of shear stress were introduced using Hanks' based, enzyme-free cell dissociation buffer (Invitrogen, Carlsbad, CA) past the adherent cells on the culture

dish. Preliminary experiments established the range of shear stresses applicable to the cell lines used in the present study (data not included). Subsequent experiments quantified each cell line's adhesion property based on the number of cells that would detach for a given period of time and a given shear stress. The number of cells detaching during the course of a single experiment were converted into percentages using the initial number of cells present in the viewable region. The following shear stress values were used 19.2, 24.0, and 28.8 dyn/cm²; each for one minute in succession for a single cell culture dish. At least three separate dishes were assayed for a given cell line to establish statistical parameters.

Results

1. Growth of anchorage-dependent and anchorage-independent HeLa cells

Growth characterization of anchorage-dependent and anchorage-independent HeLa cells was performed in bioreactors under the same set of conditions. Samples for microarray analysis were taken at the same time point, when both cell lines were in exponential growth. Both cell lines were experiencing similar environments as determined by measuring metabolic concentrations, cell density, pH, and viability. For example, both cell lines had a glucose concentration of approximately 3 g/L and a lactate concentration of approximately 1 g/L. In addition, both cells lines had a viability of at least 94%.

2. Comparing gene transcription levels in anchorage-dependent and anchorage-independent HeLa cells

Total RNA samples from anchorage-dependent and anchorage-independent HeLa cell cultures were purified, reverse transcribed, and analyzed using cDNA microarrays. Initial data analysis used GenePix Pro to visualize the hybridization on each of the 4 hybridized microarray slides. Over 8,400 of the approximately 14,000 genes spotted on the arrays were of sufficient quality to be included in the normalization procedure using the total intensity model. Following normalization, the data were filtered to identify genes with the highest and lowest expression ratios per array, defined as follows:

$$\text{Expression ratio} = \text{test sample intensity} / \text{control sample intensity} \quad \text{Eq. (1)}$$

In the present study, anchorage-independent HeLa cells were selected as the test sample whereas anchorage-dependent HeLa cells were selected as the control sample. Since there were 4 hybridized cDNA microarrays, there were 4 expression ratios per gene. An expression ratio of unity indicates equal hybridization between the two samples being assayed. Therefore, expression ratios above 1.0 are often referred to as being upregulated whereas expression ratios below 1.0 are often referred to as being downregulated.

An initial round of screening yielded approximately 3,500 genes based on consistency between slides in terms of how close the expression ratio from any one slide was to the median expression ratio calculated from all four slides. Next, the data were filtered to identify genes with expression ratios from the slides in the same direction (i.e. either upregulated or downregulated across all four slides). As a result, the number of genes selected was reduced to 667. These genes were then organized using the following clustering algorithms: self-organizing maps (SOMs), principle component analysis (PCA), and hierarchical clustering (Saeed et al., 2003, Quackenbush, 2001). PCA indicated either groups of 8 or 13 clusters were likely to form. Using this information, SOMs and hierarchical clustering were then applied to the data. By changing the parameters typically specified for either of these algorithms, new configurations were constructed and evaluated. The resulting groups were then probed to identify subsets of genes with relevance to adhesion by searching for genes closely associated with other genes known to be involved in adhesion such as integrins and cadherins. Of these

genes, the most differentially expressed were then identified, as shown in Table 1. Next, these genes were evaluated based on the known or proposed functionalities of these genes, as indicated in the literature. In the end, two genes were selected for further analysis based on their proposed functionalities, expression levels, and clustering outcomes; *siat7e* and *lama4*.

The expression of *siat7e* was higher in anchorage-independent HeLa cells than in anchorage-dependent HeLa cells, while the expression of *lama4* was lower in anchorage-independent HeLa cells than in anchorage-dependent HeLa cells. Both Figure 1 and Table 2 illustrate these expression ratios. It should be noted that although, it is common to find highly differentially expressed genes in typical microarray-related studies, in many cases it is simply a matter of what is or is not excluded. In the present study large differences in expression levels were not seen because: the two cell lines studied were very similar, care was taken to ensure the quality and quantity of RNA used for microarray analysis, and borderline spots were not considered in the subsequent analysis.

3. Verification of differentially expressed genes

As can be seen in Figure 1, the expression ratios of *siat7e* were consistently above 1 while the expression ratios for *lama4* were consistently below 1. However, the inherent variability between these slides in terms of expression ratios for the two selected genes established the need for verification. To validate the results from the cDNA microarrays, two pairs of oligonucleotide microarrays as well as a series of RT-PCR experiments were conducted. The results of these experiments are shown in Table 2 which demonstrates that the median expression levels across all three platforms are consistent with one another for several genes, including two control genes, *gapd* and *pgk1*.

4. Effects of enhancing the expression of *siat7e* and reducing the expression of *lama4* in anchorage-dependent HeLa cells

Given the transcriptional differences of *siat7e* and *lama4* between anchorage-independent and anchorage-dependent HeLa cells, as well as the results of various clustering algorithms, the expression of these genes could play a role in the adhesion and/or growth characteristics of HeLa cells. If so, then enhancing or inhibiting their expression should affect cell physiology. To evaluate the effect of *siat7e* on cell behavior, the expression of *siat7e* in anchorage-dependent HeLa cells was enhanced via transient transfection of the full-length gene. As shown in Figure 2 by comparing the image of anchorage-dependent HeLa cells (A) with the image of anchorage-dependent HeLa cells with enhanced expression of *siat7e* (B), enhancing the expression of *siat7e* caused a decrease in the percentage of viable cells attached to the surface as well as a change in cell shape for some cells from elongated, cylindrical cells to compact, spherical cells. In addition, it was observed that for a given period of time under the same growth conditions (i.e. media, temperature, etc.) the anchorage-independent HeLa cells grew to higher cell densities. (data not shown).

Reducing the transcription of *lama4* in anchorage-dependent HeLa cells by transiently transfecting the cells with gene-specific siRNA also resulted in a change illustrated in Figure 2 when comparing the image of anchorage-dependent HeLa cells (A) with the image of anchorage-dependent HeLa cells with reduced expression of *lama4* (C). In this case, the change was a 10% reduction in the percentage of attached cells. Simultaneous modifications to the expression levels of *siat7e* and *lama4* in anchorage-dependent HeLa cells did not augment these observed changes (data not shown).

5. Effects of reducing the expression of *siat7e* and enhancing the expression of *lama4* in anchorage-independent HeLa cells

Reducing the expression level of *siat7e* or enhancing the expression of *lama4* on anchorage-independent HeLa cells via transient transfections were also studied. The effect of reducing the expression of *siat7e* using gene-specific siRNA can be seen in Figures 2 and 3. Comparing the image of anchorage-independent HeLa cells (D) to the image of anchorage-independent HeLa cells with reduced expression of *siat7e* (E) in Figure 2 reveals the effects of reducing the expression of *siat7e* in anchorage-independent HeLa cells. This reduction results in an increase in the percentage of cells adhering to the surface, an increase in the percentage of cells adhering to one another (i.e. clumping), and a decrease in the percentage of cells detached from the surface. In addition, a greater number of cells appeared to elongate and take on shapes that were non-spherical, especially when attached to the surface. In order to investigate further the behavior of anchorage independent HeLa cells following downregulation of *siat7e*, an assay was devised to measure size distribution of cells and cells associated in aggregates in culture.

As seen for the control populations in Figure 3, one-half of the anchorage-independent HeLa cells existed individually (5 – 15 μm) because an individual HeLa cell is roughly 12 μm in diameter (for the narrowest portion of the cell). However, for *siat7e*-specific siRNA treated cells, the percentage of individually existing cells was reduced to one-third. In addition, there was an increase in the percentage of cells existing in larger clusters following inhibition of *siat7e*. Differences between *siat7e*-specific siRNA treated cells and control cells were statistically significant for three ranges, 5-15 μm , 61-80 μm , and 81-100 μm using a one-tailed Student's t-test with at least a 10% significance level. Indeed, the anchorage independent [*siat7e*-] cells exhibited greater clustering at all three size groupings above 60 μm .

The results of enhancing the expression of *lama4* in anchorage-independent HeLa can be seen in Figures 2 and 4. By comparing the image of anchorage-independent HeLa cells (D) and the image of anchorage-independent HeLa cells with enhanced expression of *lama4* (F) in Figure 2, it can be seen that upregulating *lama4* in anchorage-independent HeLa cells resulted in an increase in the percentage of cells adhering to the surface or clumping together, and a morphological change from spherical to more elongated shapes for those cells adhering to the surface. Shown in Figure 4 are the results of the cell size distribution assay comparing anchorage-independent HeLa and anchorage-independent HeLa with enhanced expression of *lama4*. Enhancing the expression of *lama4* in anchorage-independent HeLa cells resulted in a reduction in the percentage of cells existing individually (55% to 41%) and an increase in the percentage of cells existing in clusters; a phenomenon similar to that observed when reducing the expression of *siat7e* in anchorage-independent HeLa cells. Differences between anchorage-independent HeLa cells with inserts of *lama4* and control cells were statistically significant for two ranges; 5-15 μm and >60 μm , using a one-tailed Student's t-test with at least a 10% significance level. The clusters greater than 60 μm were not separated into individual categories since there were fewer large clusters in the anchorage independent HeLa with enhanced expression of *lama4* compared with anchorage independent HeLa with reduced expression of *siat7e*.

6. Quantifying adhesion of cells with DNA plasmid inserts

Next, the expression levels of either *siat7e* or *lama4* were altered by transfecting plasmid DNA. Each plasmid used contained the neomycin gene, conferring resistance to the compound geneticin. This resistance allowed for selection of clones expressing the plasmid. To enhance the expression of either gene, a vector containing the full-length gene sequence was used. On the other hand, to reduce the expression of either gene, a vector containing the appropriate siRNA sequence was used. Each cell line expressed either the *siat7e* or the *lama4* gene or gene-specific siRNA, constitutively. To verify the adhesion properties of these plasmid-expressing

cell lines, a shear flow chamber previously used for characterizing cellular properties was utilized. A range of shear stresses over which a clear distinction could be made between the control cell lines, anchorage-dependent HeLa and anchorage-independent HeLa was established.

Figure 5 illustrates the percentages of cells dissociating due to different shear stresses. In particular, Figure 5A shows these percentages for anchorage-dependent HeLa cells that were either unmodified, contained enhanced *siat7e* expression, or contained reduced *lama4* expression. The rate of dissociation and the percentage of cells dissociating with increasing shear stress were greater for both of these modified cell lines than for the unmodified anchorage-dependent cells, possibly indicating weakened adhesion. Similarly, Figure 5B illustrates the percentages of anchorage-independent HeLa cells dissociating. Three cell lines are shown in Figure 5B, anchorage independent HeLa cells that were either unmodified, contained reduced *siat7e* expression, or contained enhanced *lama4* expression. The percentage of cells dissociated was lower for the cells with reduced *siat7e* expression compared to the unmodified anchorage-independent HeLa cells and same cells with increasing *lama4* expression. In general, all of the anchorage-independent cell lines were less adherent than the anchorage-dependent cell lines.

By averaging multiple runs for each cell line and converting the number of dissociated cells into percentages it was possible to illustrate differences in adhesion between the different cell lines. When the expression of *lama4* was reduced in anchorage-dependent HeLa cells, the percentage of cells dissociating increased from $12\% \pm 5\%$ to $23\% \pm 5\%$. When the expression of *siat7e* was reduced in anchorage-independent HeLa cells, the percentage of cells dissociating decreased from an average of $72\% \pm 6\%$ to $53\% \pm 5\%$. The opposite pattern of modified adhesion ability was obtained for the reciprocal cases. When the expression of *lama4* was enhanced in anchorage-independent HeLa cells, the percentage of cells dissociating decreased from $72\% \pm 6\%$ to $61\% \pm 4\%$. When the expression of *siat7e* was enhanced in anchorage-dependent HeLa cells, the percentage of cells dissociating increased from $12\% \pm 5\%$ to $32\% \pm 5\%$.

Discussion

In the present study, DNA microarrays along with other genomics tools were used to identify the genes *siat7e* and *lama4* as influential to the adhesion of HeLa cells. The selection of these two genes from a broader list of differentially expressed genes was based on: proposed or known functionalities detailed in previous studies, expression levels, and clustering outcomes. Anchorage-independent HeLa cells exhibited higher expression of *siat7e* and lower expression of *lama4* relative to anchorage-dependent HeLa cells. The expression levels of these two genes were then modified *in vivo* and the resulting changes were quantified using: a microscope, a particle counter, and an attachment assay. By either enhancing the expression of *siat7e* or reducing the expression of *lama4* in anchorage-dependent HeLa cells, a reduction in cellular adhesion was observed. Conversely, by either reducing the expression of *siat7e* or enhancing the expression of *lama4* in anchorage-independent HeLa cells, enhanced aggregation and cellular adhesion were observed.

These findings on the impact of *siat7e* and *lama4* on cellular adhesion are consistent with prior studies on the functions of these genes. The gene *lama4* encodes laminin $\alpha 4$, a secreted protein consisting of 1,816 amino acids and detected in various tissues including the skin, placenta, heart, lung, skeletal muscle, and pancreas (Richards et al., 1996). Laminins, a family of secreted glycoproteins, are a major component of the basal lamina; a layer found beneath epithelial cells and surrounding connective tissue with functional relevance to a variety of cellular properties such as morphology, polarity, adhesion, and migration (Haralson and Hassell, 1995; Gonzalez

et al., 2002; Kalmykova et al., 2002; Colognato and Yurchenco, 2000). Each laminin molecule consists of three distinct subunits (α -, β -, and γ -chains) making it a heterotrimer (Colognato and Yurchenco, 2000). Laminin $\alpha 4$ acts as a ligand for several integrin heterodimers including $\alpha v \beta 3$ and $\alpha 3 \beta 1$, both of which are involved in angiogenesis and endothelial cell interaction (Gonzalez et al., 2002; Reddy and Kalraiya, 2006).

Previous studies have explored the functionality of different laminins in a variety of settings with specific interactions in mind. In the context of wound healing, migration, and adhesion, laminin 2 and laminin 4 were deemed good substrates for human keratinocytes; cells found in the skin, hair, and nails that synthesize keratin (Kalmykova et al., 2002). In addition, both laminins support epidermal growth factor-mediated migration and in relation to other laminins, have a higher number of cell surface receptors (Kalmykova et al., 2002). Similarly, coating various surfaces with peptide motifs shared by laminins such as Ile-Lys-Val-Ala-Val (IKVAV) increased cellular adhesion to those surfaces (Lin et al., 2006). Laminin also appears to support the growth of mouse embryonic stem cells by contributing to the adsorption of serum-derived proteins and enhancing the stability of resulting cellular assemblies (Brynda et al., 2005). Thus, the increase in expression of laminin in anchorage-dependent HeLa cells is entirely consistent with the role this protein plays in cellular adhesion processes.

In contrast, the expression level of *siat7e* in anchorage-independent HeLa cells was more than twice the expression level in anchorage-dependent HeLa cells. *Siat7e* encodes a type II membrane protein, α -N-acetylgalactosaminide $\alpha 2,6$ sialyltransferase V (ST6GalNAc V), found in the Golgi apparatus. This protein is responsible for the synthesis of GD1 α (disialoganglioside) from GM1b (monosialoganglioside) which involves the addition of a N-acetylneuraminic acid (NeuAc) onto the N-acetylgalactosamine (GalNAc) residue of the acceptor molecule, NeuAc $\alpha 2,3$ Gal $\beta 1,3$ GalNAc $\beta 1,4$ Gal $\beta 1,4$ Glc $\beta 1$ -Ceramide where Gal represents Galactose and Glc represents Glucose (Ikehara et al., 1999; Okajima et al., 1999; Taki et al., 1997; Yu et al., 2004; Yanagisawa et al., 2004). As a result, the protein ST6GalNAc V is also referred to as GD1 α synthase (Sawada et al., 1999; Taki et al., 1997; Ito et al., 2003). A ganglioside consists of a glycosphingolipid (glycolipids containing sphingosine) with one or more sialic acids; predominantly NeuAc (Wiegandt, 1985). Gangliosides are known to be critical to numerous cellular functions including cell-cell interactions, tissue differentiation, and cell adhesion (Liu et al., 2004; Okajima et al., 1999; Tsuchida et al., 2003; Hakomori, 1981). The presence of sialic acids and the nature of their linkages on a ganglioside can be especially relevant to biological functions (Okajima et al., 1999).

More recently it was uncovered that ST6GalNAc V along with ST6GalNAc VI have another sialyltransferase function; the addition of a second NeuAc onto the N-acetylglucosamine (GlcNAc) residue of monosialyl lactotetraosylceramide (Lc4) with an $\alpha 2,6$ linkage (Tsuchida et al., 2003). Monosialyl Lc4 (NeuAc $\alpha 2,3$ Gal $\beta 1,3$ GlcNAc $\beta 1,3$ Gal $\beta 1,4$ Glc $\beta 1$ -Ceramide) serves as a precursor for the synthesis of either monosialyl or disialyl Lewis x (Le^a). Both monosialyl Le^a and disialyl Le^a are thought to play important roles in a variety of cellular processes including oncogenesis (Magnani, 2004; Itai et al., 1991; Julien et al., 2001).

Consistent with these studies, the gene expression of ST6GalNAc VI was found to be downregulated in a number of colon carcinoma cell lines (Miyazaki et al., 2004; Suzuki et al., 2006). Furthermore, transfection of several cell lines with ST6GalNAc VI revealed an increase in disialyl Le^a expression along with a reduction in monosialyl Le^a expression (Tsuchida et al., 2003; Miyazaki et al., 2004; Suetake et al., 2003). Furthermore, the transfection altered the adhesive properties of the cells by lowering their adhesion to E-selectin while increasing their binding to sialic acid binding Ig-like lectin 7 (Siglec-7) (Miyazaki et al., 2004). Therefore, altering the sialic acid content on a glycolipid by modifying the expression of this family of

sialyltransferases can confer significant changes in cellular adhesion. Although, both ST6GalNAc V and ST6GalNAc VI share a number of features including sequence motifs, overall structure, and function, only ST6GalNAc V was found to be upregulated in the current study for anchorage-independent HeLa cell lines (Patel and Balaji, 2006). Nonetheless, a change in expression of *siat7e* in the attachment-independent cell lines is consistent with the role it plays in cellular adhesion processes. It would be interesting to determine the expression levels of binding partners to these glycolipids on the surface of HeLa cell lines.

Thus, changes of the expression levels of *lama4* and *siat7e* are consistent with the evolution of different adhesion properties for the two HeLa cell lines evaluated in the current study. Furthermore, it has been suggested that sialyltransferase genes can be subject to methylation silencing (Sakakura et al., 2005; Miyazaki et al., 2004; Reddy and Kalraiya, 2006; Brynda et al., 2005). As such, it is conceivable a similar epigenetic transformation may be occurring in order for HeLa cells to adapt to suspension conditions. Finally, there is the potential for synergistic effects between laminins and sialyltransferases, although the current study did not reveal any significant alteration in the adhesion properties of the HeLa cells when the two genes were modified in concert (Wang et al., 2006; Suzuki et al., 2002).

References

- Abulencia JP, Gaspard R, Healy ZR, Gaarde WA, Quackenbush J, Konstantopoulos K. Shear-induced cyclooxygenase-2 via a JNK2/c-Jun-dependent pathway regulates prostaglandin receptor expression in chondrocytic cells. *J Biol Chem* 2003;278:28388–28394. [PubMed: 12743126]
- Bleckwenn NA, Bentley WE, Shiloach J. Evaluation of production parameters with the vaccinia virus expression system using microcarrier attached HeLa cells. *Biotechnol Prog* 2005;21:554–561. [PubMed: 15801798]
- Brynda E, Pachernik J, Houska M, Pientka Z, Dvorak P. Surface immobilized protein multilayers for cell seeding. *Langmuir* 2005;21:7877–7783. [PubMed: 16089395]
- Burke HB. Discovering Patterns in Microarray Data. *Mol Diagn* 2000;5:349–357. [PubMed: 11172499]
- Butte A. The Use and Analysis of Microarray Data. *Nat Rev Drug Discov* 2002;1:951–960. [PubMed: 12461517]
- Chu L, Blumentals I, Maheshwari G. Production of recombinant therapeutic proteins by mammalian cells in suspension culture. *Methods Mol Biol* 2005;308:107–121. [PubMed: 16082030]
- Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. *Dev Dyn* 2000;218:213–234. [PubMed: 10842354]
- Conway T, Schoolnik GK. Microarray expression profiling: capturing a genome. *Mol Microbiol* 2003;47:879–889. [PubMed: 12581346]
- Crowther DJ. Applications of microarrays in the pharmaceutical industry. *Curr Opin Pharmacol* 2002;2:551–554. [PubMed: 12324258]
- Gonzalez AM, Gonzales M, Herron GS, Nagavarapu U, Hopkinson SB, Tsuruta D, Jones JC. Complex interactions between the laminin alpha 4 subunit and integrins regulate endothelial cell behavior in vitro and angiogenesis in vivo. *Proc Natl Acad Sci USA* 2002;99:16075–16080. [PubMed: 12454288]
- Hakomori S. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu Rev Biochem* 1981;50:733–764. [PubMed: 7023369]
- Haralson, MA.; Hassell, JR. Extracellular matrix-A practical approach. Oxford University Press; New York: 1995. p. 99-101.
- Hatzimanikatis V, Lee KH. Dynamical analysis of gene networks requires both mRNA and protein expression information. *Metab Eng* 1999;1:275–281. [PubMed: 10937820]
- Hegde P, Qi R, Abernathy K, Gay C, Dharap S, Gaspard R, Hughes JE, Snesrud E, Ikehara Y, Shimizu N, Kono M, Nishihara S, Nakanishi H, Kitamura T, Narimatsu H, Tsuji S, Tatematsu M. A novel glycosyltransferase with a polyglutamine repeat; a new candidate for GD1a synthase (ST6GalNAc V). *FEBS Lett* 1999;463:92–96. [PubMed: 10601645]

- Itai S, Nishikata J, Yoneda T, Ohmori K, Yamabe H, Arai S, Tobe T, Kannagi R. Tissue distribution of sialyl 2-3 and 2-6 Lewis a antigens and the significance of serum 2-3/2-6 sialyl Lewis a antigen ratio for the differential diagnosis of malignant and benign disorders of the digestive tract. *Cancer* 1991;67:1576–1587. [PubMed: 2001547]
- Ito H, Ishida H, Collins BE, Fromholt SE, Schnaar RL, Kiso M. Systematic synthesis and MAG-binding activity of novel sulfated GM1b analogues as mimics of Chol-1 (alpha-series) gangliosides: highly active ligands for neural siglecs. *Carbohydr Res* 2003;338:1621–1639. [PubMed: 12873419]
- Julien S, Krzewinski-Recchi MA, Harduin-Lepers A, Gouyer V, Huet G, Bourhis XL, Delannoy D. Expression of Sialyl-Tn antigen in breast cancer cells transfected with the human CMP-Neu5Ac: GalNAc a2, 6 sialyltransferase (ST6GalNAc I) cDNA. *Glycoconj J* 2001;18:883–893. [PubMed: 12820722]
- Kalmykova NV, Cherepanova OA, Gorelik IuV, Voronkina IV, Blinova MI, Pinaev GP. Various effects of laminin-1 and laminin-2/4 on adhesion and migration of cultured human keratinocytes. *Tsitologiya* 2002;44:792–798. [PubMed: 12506671]
- Karu TI, Pyatibrat LV, Kalendo GS. Cell attachment modulation by radiation from a pulsed light diode ($\lambda = 820$ nm) and various chemicals. *Lasers Surg Med* 2001;28:227–236. [PubMed: 11295757]
- Lee N, Quackenbush J. A concise guide to cDNA microarray analysis. *Biotechniques* 2000;29:548–550. 552–554. [PubMed: 10997270] 556 passim
- Lin X, Takahashi K, Liu Y, Zamora PO. Enhancement of cell attachment and tissue integration by a IKVAV containing multi-domain peptide. *Biochim Biophys Acta* 2006;1760:1403–1410. [PubMed: 16860485]
- Liu Y, Li R, Ladisch S. Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. *J Biol Chem* 2004;279:36481–36489. [PubMed: 15215248]
- Lum AM, Huang J, Hutchinson CR, Kao CM. Reverse engineering of industrial pharmaceutical-producing actinomycete strains using DNA microarrays. *Metab Eng* 2004;6:186–196. [PubMed: 15256208]
- Magnani JL. The discovery, biology, and drug development of sialyl Lea and sialyl Lex. *Arch Biochem Biophys* 2004;426:121–131.
- Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. *Nat Rev Cancer* 2002;2:315–319. [PubMed: 12001993]
- Mise-Omata S, Obata Y, Iwase S, Mise N, Doi TS. Transient strong reduction of PTEN expression by specific RNAi induces loss of adhesion of the cells. *Biochem Biophys Res Commun* 2005;328:1034–1042. [PubMed: 15707982]
- Miyazaki K, Ohmori K, Izawa M, Koike T, Kumamoto K, Furukawa K, Ando T, Kiso M, Yamaji T, Hashimoto Y, Suzuki A, Yoshida A, Takeuchi M, Kannagi R. Loss of disialyl Lewis(a), the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) associated with increased sialyl Lewis(a) expression on human colon cancers. *Cancer Res* 2004;64:4498–4505. [PubMed: 15231659]
- Nakanishi T, Oka T, Akagi T. Recent Advances in DNA Microarrays. *Acta Medica Okayama* 2001;55:319–328. [PubMed: 11779093]
- Okajima T, Fukumoto S, Ito H, Kiso M, Hirabayashi Y, Urano T, Furukawa K. Molecular cloning of brain-specific GD1a synthase (ST6GalNAc V) containing CAG/Glutamine repeats. *J Biol Chem* 1999;274:30557–30562. [PubMed: 10521438]
- Patel RY, Balaji PV. Identification of linkage-specific sequence motifs in sialyltransferases. *Glycobiology* 2006;16:108–116. [PubMed: 16207893]
- Quackenbush J. Computational Analysis of Microarray Data. *Nat Rev Genet* 2001;2:418–427. [PubMed: 11389458]
- Reddy BV, Kalraiya RD. Sialylated beta1,6 branched N-oligosaccharides modulate adhesion, chemotaxis and motility of melanoma cells: Effect on invasion and spontaneous metastasis properties. *Biochim Biophys Acta* 2006;1760:1393–1402. [PubMed: 16806716]
- Richards A, Al-Imara L, Pope FM. The complete cDNA sequence of laminin alpha 4 and its relationship to the other human laminin alpha chains. *Eur J Biochem* 1996;238:813–821. [PubMed: 8706685]
- Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z,

- Vinsavich A, Trush V, Quackenbush J. TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* 2003;34:374–378. [PubMed: 12613259]
- Sakakura C, Hasegawa K, Miyagawa K, Nakashima S, Yoshikawa T, Kin S, Nakase Y, Yazumi S, Yamagishi H, Okanoue T, Chiba T, Hagiwara A. Possible involvement of RUNX3 silencing in the peritoneal metastases of gastric cancers. *Clin Cancer Res* 2005;11:6479–6488. [PubMed: 16166423]
- Sawada N, Ishida H, Collins BE, Schnaar RL, Kiso M. Ganglioside GD1 alpha analogues as high-affinity ligands for myelin-associated glycoprotein (MAG). *Carbohydr Res* 1999;316:1–5. [PubMed: 10420586]
- Simon R, Radmacher MD, Dobbin K. Design of studies using DNA microarrays. *Genet Epidemiol* 2002;23:21–26. [PubMed: 12112246]
- Springer EL, Hackett AJ, Nelson-Rees WA. Alteration of the cell membrane architecture during suspension and monolayer culturing. *Int J Cancer* 1976;407–415. [PubMed: 1254361]
- Suetake K, Liour SS, Tencmnao T, Yu RK. Expression of gangliosides in an immortalized neural progenitor/stem cell line. *J Neurosci Res* 2003;74:769–776. [PubMed: 14635228]
- Suzuki O, Nozawa Y, Abe M. The regulatory roles of cell surface sialylation and N-glycans in human B cell lymphoma cell adhesion to galectin-1. *Int J Oncol* 2006;28:155–160. [PubMed: 16327992]
- Suzuki O, Nozawa Y, Kawaguchi T, Abe M. UDP-GlcNAc2-epimerase regulates cell surface sialylation and cell adhesion to extracellular matrix in Burkitt's lymphoma. *Int J Oncol* 2002;20:1005–1011. [PubMed: 11956597]
- Taki T, Ishikawa D, Ogura M, Nakajima M, Handa S. Ganglioside GD1alpha functions in the adhesion of metastatic tumor cells to endothelial cells of the target tissue. *Cancer Res* 1997;57:1882–1888. [PubMed: 9157980]
- Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM. Systematic determination of genetic network architecture. *Nat Genet* 1999;22:281–285. [PubMed: 10391217]
- Tsuchida A, Okajima T, Furukawa K, Ando T, Ishida H, Yoshida A, Nakamura Y, Kannagi R, Kiso M, Furukawa K. Synthesis of disialyl Lewis a (Lea) structure in colon cancer cell lines by a sialyltransferase, ST6GalNAc VI, responsible for the synthesis of alpha-series gangliosides. *J Biol Chem* 2003;278:22787–22794. [PubMed: 12668675]
- Wang XY, Lin SQ, Li JW, Kemmner W, Ding YQ. Effect of cell surface sialic acid and their linkages on adhesion of mammary carcinoma cells. *Nan Fang Yi Ke Da Xue Xue Bao* 2006;26:742–746. [PubMed: 16793589]
- Wiegandt, H. Gangliosides. In: Wiegandt, H., editor. *Glycolipids, New Comprehensive Biochemistry*. 10. Elsevier; Amsterdam: 1985. p. 199–260.
- Xiang Z, Yang Y, Ma X, Ding W. Microarray expression profiling: Analysis and applications. *Curr Opin Drug Discov Devel* 2003;6:384–395.
- Yanagisawa M, Liour SS, Yu RK. Involvement of gangliosides in proliferation of immortalized neural progenitor cells. *J Neurochem* 2004;91:804–812. [PubMed: 15525334]
- Yu RK, Bieberich E, Xia T, Zeng G. Regulation of ganglioside biosynthesis in the nervous system. *J Lipid Res* 2004;45:783–793. [PubMed: 15087476]
- Zhu M, Gong Y, Frenkel GD. Studies on the mechanism of the selenite-induced decrease in cell attachment: effect of selenite on the levels of fibronectin receptor (a5b1 integrin) mRNAs. *Biol Trace Elem Res* 1998;62:123–134. [PubMed: 9676878]
- Zhu Y, Wu Z, Tang Z, Lu Z. HeLa cell adhesion on various collagen-grafted surfaces. *J Proteome Res* 2002;1:559–562. [PubMed: 12645624]

Figure 1.

Median expression ratios for two specific genes from four different microarray slides. The error bars indicate the range of values observed based on several different spots for each gene.

Figure 2.

Pictures of different cell lines at a magnification of 10X

A - anchorage-dependent HeLa

B - anchorage-dependent with enhanced expression of *siat7e*

C - anchorage-dependent with reduced expression of *lama4*

D - anchorage-independent HeLa

E - anchorage-independent with reduced expression of *siat7e*

F - anchorage-independent with enhanced expression of *lama4*

Figure 3.
Distribution of clusters 5 - 100 μm in anchorage-independent HeLa cells with and without siRNA specific for *siat7e*.

Figure 4. Distribution of clusters 5-60 μm in anchorage-independent HeLa cells with and without the *lama4* gene insert.

A

B

Figure 5.

Cell dissociation as a function of shear stress.

5A. anchorage-dependent HeLa cells: unmodified cells, cells with enhanced expression of *siat7e* (sialyltransferase +), and cells with reduced expression of *lama4* (laminin-)5B. anchorage-independent HeLa cells: unmodified cells, cells with enhanced expression of *lama4* (laminin +), and cells with reduced expression of *siat7e* (sialyltransferase -)

Table 1

Proposed or known functionalities for several differentially expressed genes

Gene Symbol	Functionality of Gene Product	Maximum Expression Ratio	Minimum Expression Ratio
<i>siat7e</i>	Involved in glycosylation Exhibits higher activity with glycolipids than with glycoproteins Type II membrane protein (Golgi) Complex glycoprotein that mediates the adhesion, migration and organization of cells into tissues during embryonic development	3.63	1.54
<i>lama4</i>	by interacting with other extra cellular matrix components Member of family of extra cellular matrix glycoproteins	1.05	0.22
<i>cpb2</i>	Enzyme that hydrolyzes C-terminal peptide bonds (cleaving basic amino residues) and is activated by trypsin Downregulates fibrinolysis following thrombin activation	4.32	1.72
<i>panx3</i>	Member of the innexin family Structural component of gap junctions Member of the bone morphogenetic protein family	0.81	0.175
<i>gdf1</i>	Secreted protein that may mediate cell differentiation events	3.68	1.25
<i>endogl1</i>	May regulate cell growth Member of DNA/RNA non-specific endonuclease family	1.20	0.19
<i>rheb</i>	Binds GTP and exhibits intrinsic GTPase activity (by similarity) Belongs to the small GTPase superfamily	4.05	1.92

Table 2

Median expression ratios for specific genes across varying platforms; cDNA microarrays, oligonucleotide microarrays, and RT-PCR

Gene Symbol	Gene Title	cDNA	Oligonucleotide	RT-PCR
<i>siat7e</i>	Alpha-N-acetylgalactosaminide alpha-2 6-sialyltransferase V	2.01	2.35	2.21
<i>lama4</i>	Laminin alpha-4 chain precursor	0.73	0.67	0.76
<i>gapd</i>	Glyceraldehyde-3-phosphate dehydrogenase	1.03	0.97	1.08
<i>pgk1</i>	Phosphoglycerate kinase 1	0.98	1.12	0.94