MUC4, a Multifunctional Transmembrane Glycoprotein, Induces Oncogenic Transformation of NIH3T3 Mouse Fibroblast Cells

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Abstract

Numerous studies have established the association of MUC4 with the progression of cancer and metastasis. An aberrant expression of MUC4 is reported in precancerous lesions, indicating its early involvement in the disease process; however, its precise role in cellular transformation has not been explored. MUC4 contains many unique domains and is proposed to affect cell signaling pathways and behavior of the tumor cells. In the present study, to decipher the oncogenic potential of MUC4, we stably expressed the MUC4 mucin in NIH3T3 mouse fibroblast cells. Stable ectopic expression of MUC4 resulted in increased growth, colony formation, and motility of NIH3T3 cells in vitro and tumor formation in nude mice when cells were injected s.c. Microarray analysis showed increased expression of several growth-associated and mitochondrial energy production–associated genes in MUC4-expressing NIH3T3 cells. In addition, expression of MUC4 in NIH3T3 cells resulted in enhanced levels of oncoprotein ErbB2 and its phosphorylated form (pY1248–ErbB2). In conclusion, our studies provide the first evidence that MUC4 alone induces cellular transformation and indicates a novel role of MUC4 in cancer biology.

Introduction

MUC4 is a member of the membrane-bound mucin family (1, 2). It was cloned from a human tracheobronchial cDNA library and pancreatic tumor cell line and its complete genomic organization has been established (25 exons/introns over 65 kb; ref. 3). MUC4 is synthesized as a single polypeptide chain of ~930 kDa and hypothesized to be cleaved at a GDPH proteolytic cleavage site generating two subunits: the mucin type subunit MUC4α and a transmembrane subunit MUC4β. MUC4α possesses three putative functional domains: tandem repeat, nidogen-like, and adhesion-associated domain in MUC4 and other proteins, whereas MUC4β has three epidermal growth factor–like domains and a short cytoplasmic tail (3).

MUC4 is normally expressed by the luminal epithelial cells of the stomach, colon, lung, trachea, cervix, and prostate (4, 5), although it is not or minimally expressed by gall bladder, biliary epithelial cells, intrahepatic bile ducts, liver, and pancreas (4, 6, 7). An overexpression of MUC4 is, however, observed in pancreatic, lung, breast, colon, and ovarian malignancies, suggesting its pathologic significance (6, 8–10). Furthermore, the association of MUC4 with the poor prognosis of the pancreatic, lung, and bile duct cancer patients has also been reported (11–13). Using MUC4 knockdown and overexpression cell models, we have shown that MUC4 potentiates pancreatic tumor cell growth and metastasis by altering the behavioral properties of the tumor cells (14–16).

Importantly, our recent studies have revealed that MUC4 interacts with the receptor tyrosine kinase HER2 and regulates its expression by posttranslational mechanisms (17). HER2 is an established oncoprotein and is involved in growth and malignant properties of the cancer cells (18). An aberrant expression of MUC4 in pancreatic cancer is detected early (i.e., in precancerous lesions) and correlates with the disease advancement (19, 20). All these findings indicate that MUC4 may play an important role in the early and late events of cancer progression.

In the present study, we have carried out a set of experiments to define the role of MUC4 in oncogenic transformation. MUC4 was ectopically overexpressed in NIH3T3 mouse fibroblast cells by stable transfection and its effect on the cellular phenotype was determined by performing in vitro and in vivo functional assays. Ectopic expression of MUC4 in NIH3T3 cells resulted in increased growth, colony formation, and motility of the cells. Furthermore, MUC4-expressing NIH3T3 cells spontaneously formed tumors in nude mice in majority of cases (73%) when injected s.c. An enhanced expression of ErbB2, its phosphorylated form (pY1248–ErbB2), and phosphorylated extracellular signal-regulated kinase (pERK) was observed in MUC4-expressing NIH3T3 cells. Additionally, ectopic expression of MUC4 was found to alter the expression of several growth-associated and mitochondrial energy production–associated genes. Together, all these observations support a role of MUC4 in cancer pathogenesis and provide first evidence for its oncogenic action.

Materials and Methods

Cell culture and transfection. NIH3T3 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin). Cells were grown at 37°C with 5% CO2 in a humidified atmosphere. pSecTagC-MUC4 plasmid, previously designed in our lab (16), was used for attaining the ectopic expression of MUC4 in NIH3T3 mouse fibroblast cells by stable transfection using FuGENE6 Transfection Reagent (Roche Diagnostics). Cells were also transfected with empty pSecTagC vector to obtain a control population. The zeocin-resistant colonies were isolated by the ring cloning method, expanded, and maintained in medium supplemented with 400 μg/ml zeocin (Invitrogen).

Immunoblot assay. The NIH3T3-derived clones were processed for protein extraction and Western blotting using standard procedures. Cell lysates were prepared as described previously (15). Protein concentrations were determined using a Bio-Rad detergent-compatible protein estimation
kit. For MUC4, the proteins (20 μg) were resolved by electrophoresis on a 2% SDS-agarose gel under reducing conditions. For β-actin, ErbB2, pY1248-ErbB2, and ERK1/2, SDS-PAGE (10%) was performed under similar conditions. Resolved proteins were transferred onto the polyvinylidene difluoride (PVDF) membrane and blocked in 5% nonfat milk in PBS for 2 h and subjected to the standard immunodetection procedure using specific antibodies. For β-actin immunodetection, anti-β-actin mouse monoclonal antibody (mAb; Sigma) in dilution of 1:2,000 (used as internal control) was used; for MUC4 immunodetection, anti-MUC4 mouse mAb (8G7, generated in our laboratory) in dilution of 1:1,000 was used. For ErbB2, pY1248-ErbB2, ERK, and pERK immunodetection, anti-ErbB2 (Santa Cruz Biotechnology), anti-pY1248-ErbB2 (Upstate), anti-ERK1/2 (Santa Cruz Biotechnology), and anti-pERK1/2 (Cell Signaling Technology) rabbit polyclonal antibodies in dilution of 1:1,000 were used. The membranes were incubated for 4 h at room temperature followed by six 10-min washes in TBST [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, and 0.05% Tween 20]. Further, the membranes were incubated in horseradish peroxidase (HRP)–conjugated secondary antibodies (diluted at 1:2,000 in PBS; Amersham Biosciences) for 1 h at room temperature followed by six washes in TBST. The blots were processed with enhanced chemiluminescence kit (Amersham Biosciences), and the signal was detected by exposing the processed blots to X-ray films (Biomax Films).

Confocal immunofluorescence microscopy. For immunofluorescence staining, cells were grown at low density on sterilized coverslips for 20 h. Cells were first washed with 0.1 mol/L HEPES containing Hank’s buffer and then fixed in ice-cold methanol at −20°C for 2 min. Methanol-fixed cells were blocked in 10% goat serum containing 0.05% Tween 20 for 30 min at room temperature for nonspecific blocking followed by incubation with the anti-MUC4 mAb (8G7), diluted (1:100) in PBS, for 90 min at room temperature. Cells were washed four to five times for 5 min with PBS containing 0.05% Tween 20 (PBS-T) and then incubated with FITC-conjugated goat anti-mouse secondary antibodies (Jackson Immunoresearch Laboratories, Inc.) for 60 min. Cells were then fixed in ice-cold methanol for 10 min. The slides were incubated with a Diff-Quick cell staining kit (Dade Behring, Inc.). Cells in 10 random fields of view at ×100 magnification were counted and expressed as the average number of cells per field of view.

Western blot and confocal immunofluorescence analyses of MUC4 expression in empty vector–transfected and MUC4-transfected NIH3T3 cells. A, a total of 20 μg protein from each cell extract was resolved by electrophoresis on a 2% SDS-Agarose gel, transferred to PVDF membrane, and incubated with anti-MUC4 mAb (8G7). The membrane was then probed with HRP-labeled goat anti-mouse immunoglobulin. Immunoblot of β-actin, obtained from 10% SDS-PAGE, was used as an internal control. The signal was detected using an electrochemiluminescence reagent kit. B, cells were grown at low density on sterilized coverslips and processed for the immunofluorescence procedure after fixation in methanol for 10 min. Slides were incubated with MUC4 mAb (8G7) followed by FITC-conjugated secondary antibody and were observed under a Zeiss confocal laser-scanning microscope.

Figure 1. Western blot and confocal immunofluorescence analyses of MUC4 expression in empty vector–transfected and MUC4-transfected NIH3T3 cells. A, a total of 20 μg protein from each cell extract was resolved by electrophoresis on a 2% SDS-Agarose gel, transferred to PVDF membrane, and incubated with anti-MUC4 mAb (8G7). The membrane was then probed with HRP-labeled goat anti-mouse immunoglobulin. Immunoblot of β-actin, obtained from 10% SDS-PAGE, was used as an internal control. The signal was detected using an electrochemiluminescence reagent kit. B, cells were grown at low density on sterilized coverslips and processed for the immunofluorescence procedure after fixation in methanol for 10 min. Slides were incubated with MUC4 mAb (8G7) followed by FITC-conjugated secondary antibody and were observed under a Zeiss confocal laser-scanning microscope.
data were represented as the average of the two independent experiments with the SD of the average indicated.

Oligonucleotide array gene expression analysis. Mouse oligonucleotide array containing probes for ~10,800 genes was constructed at the Microarray Core Facility of University of Nebraska Medical Center. Total RNA was isolated from MUC4 and empty vector–transfected NIH3T3 cells by RNeasy Mini kit (Qiagen). Spotted microarrays were used to determine differential gene expression between NIH3T3/MUC4 and NIH3T3/pSecTag samples. The design had NIH3T3/MUC4 and NIH3T3/pSecTag samples competitively hybridized to three arrays. To generate the fluorescently labeled, single-stranded cDNA target, total RNA (750 ng) was reverse transcribed to generate cDNA, followed by in vitro transcription to generate amino-allyl (aRNA) using the Amino Alkyl Message Amp kit (Ambion). aRNA (5 μg) was coupled with either CY5 or CY3 dye as per the manufacturer’s suggestion, mixed, and cohybridized (in 20 μL of Ambion hybridization buffer) to microarray slides for 16 h at 42 °C. The slides were washed per the manufacturer’s instructions and scanned using an Axon 4000b scanner to generate .tiff images. The images were extracted using GenePix software and resultant .GPR files were used for downstream analysis. Differentially expressed genes were identified using BRB Array Tools developed by Dr. Richard Simon and Amy Peng (Biometric Research Branch, National Cancer Institute, NIH, Bethesda, MD).

Several filters and normalization were applied before analysis. Spots were excluded if both the red and green channels had values <100, and if only one of the red or green channels was <100, it was increased to the threshold of 100. Median background was subtracted and log_2 transformation was applied to all ratios. Normalization was then done to “center” each array using lowess smoother and genes were excluded if any of the spots were missing or filtered out for any of the samples. Random-variance paired t tests were used to determine which genes are differentially expressed between NIH3T3/MUC4 and NIH3T3/pSecTag samples, comparing the log red (NIH3T3/MUC4) and green (NIH3T3/pSecTag) channel intensities. The random-variance paired t test allows sharing information among genes about variation without assuming that all genes have the same variance, which gives a more accurate estimate of the variability when sample sizes are small. A significance level of 0.001 was selected to help limit the false discovery rate due to multiple comparisons.

Quantitative reverse transcription-PCR. Total RNA (2 μg) from each of the derived NIH3T3 cell lines was reverse transcribed using the first-strand cDNA synthesis kit (Perkin-Elmer) and oligo-d(T) primers according to the manufacturer’s instructions. Real-time PCR amplifications were carried out with 100 ng of first-strand cDNA in 10-μL reaction volumes. The reaction mixture was subjected to a two-step cyclic program (95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min) as per the manufacturer’s protocol on ABI 7500 sequence detection system (Applied Biosystems) with SYBR chemistry. Predesigned PCR primers for Tln, Sna, Nek6, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from a commercial source (SuperArray Biosciences Corp.).
Relative fold difference in gene expression was calculated by ΔΔCt method (22) using GAPDH as a normalization control.

**Results**

**Constitutive overexpression of MUC4 in mouse fibroblast NIH3T3 cells.** To investigate the transforming ability of MUC4, mouse fibroblast NIH3T3 cells that do not express endogenous Muc4 were transfected with human MUC4 expression plasmid (pSecTag-MUC4; ref. 16) or empty pSecTag vector (as a control) and stable cell clones were selected. The clones were expanded and screened for MUC4 expression by immunoblot analysis. Two clones that exhibited high level of MUC4 (Fig. 1A) were pooled together and further characterized by confocal analysis (Fig. 1B). The pooled populations of MUC4-transfected (NIH3T3/MUC4) and empty vector–transfected (NIH3T3/pSecTag) cells were monitored for 1 to 2 months for stable expression and used to study the oncogenic potential of MUC4 and its effect on cellular phenotype by performing in vitro and in vivo functional assays.

**Ectopic expression of human MUC4 enhances the growth kinetics and clonogenicity of NIH3T3 cells.** To determine whether MUC4 influences the growth of NIH3T3 cells, NIH3T3/MUC4 and NIH3T3/pSecTag cells were seeded at low density (25 × 10^3 per well of six-well plate) and growth rates were determined by cell counting at different time intervals (Fig. 2A). NIH3T3/MUC4 cells showed an enhanced growth rate compared with the empty vector–transfected (NIH3T3/pSecTag) cells. Population doubling times were calculated from the growth curve during the exponential phase (192–240 hours). NIH3T3/MUC4 cells displayed a significant decrease (P < 0.05) in doubling time compared with the control cells. NIH3T3/MUC4 cells had a doubling time of 37 hours, whereas it was recorded 44 hours for the control cells (Fig. 2B). To assess the effects of MUC4 expression on the plating efficiency of NIH3T3 cells, NIH3T3/MUC4 and NIH3T3/pSecTag cells were analyzed by colony formation assay. Cells were seeded at very low density (2.5 × 10^3 per 10-cm dish), and after 2 weeks, cells were fixed and stained with 0.1% crystal violet and representative photographs were taken. NIH3T3/MUC4 cells showed significantly enhanced colony formation when compared with the vector-transfected cells (Fig. 2C).

**MUC4 expression enhances motility of NIH3T3 cells.** To examine the effect of MUC4 expression on NIH3T3 cells on behavioral properties, a motility assay was performed by using uncoated porous membranes of 8.0-mm pore diameter. The number of cells that migrated to the lower surface of the porous membrane under chemoattractive stimulus of FBS in the lower chamber was ~2-fold greater in MUC4-expressing NIH3T3/MUC4 cells in comparison with control NIH3T3/pSecTag cells (Fig. 2D). These data indicated that expression of MUC4 enhanced motility in NIH3T3 cells.

**Expression of MUC4 induces oncogenic transformation of NIH3T3 cells.** To test the hypothesis that MUC4 plays a role in cellular transformation and potentiation of tumor development, we examined the tumorigenic potential of NIH3T3/MUC4 and NIH3T3/pSecTag cells in vivo. A total of 30 immunodeficient mice were injected s.c. with NIH3T3/MUC4 and NIH3T3/pSecTag cells (1 × 10^6) and tumor growth was measured twice a week in two independent experiments. Tumor volumes were calculated from bidimensional measurements at each time point and mice were sacrificed on day 36 after injection. Results were expressed as differences in tumor-free survival and tumor volumes were also compared between these two groups. The tumor-free survival experience was significantly decreased in NIH3T3/MUC4 cell–injected mice (P < 0.001). The mean tumor-free survival time for NIH3T3/MUC4 mice (n = 15) was only 17.6 days compared with 34.7 days for control animals (n = 15; Fig. 3A). Only 7% of mice injected with MUC4-expressing NIH3T3 cells showed tumor-free survival on 36th day of implantation of cells compared with 73%

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**Figure 3.** MUC4 induces tumorigenicity in NIH3T3 cells. NIH3T3/MUC4 and NIH3T3/pSecTag cells (1 × 10^6) were injected s.c. into the nude mice. Tumor volumes were calculated from bidimensional measurements at each time point. Results were expressed as differences in tumor-free survival and tumor volumes between mice injected with NIH3T3/MUC4 and NIH3T3/pSecTag cells; each group includes 15 mice per group. A, differences in tumor-free survival were measured using Kaplan-Meier plots and survival curves were compared using the log-rank test. B, tumor volumes were compared between these groups. Points, mean; bars, SE.
of mice injected with control cells. Furthermore, tumor volumes were significantly increased in mice injected with NIH3T3/MUC4 cells compared with the mice injected with empty vector–transfected NIH3T3/pSecTag cells (P < 0.05; Fig. 3B). No observable metastasis, however, was observed in any of the control-injected or NIH3T3/MUC4 cell–injected mice.

**MUC4 expression enhances the expression of ErbB2 and its downstream signaling in NIH3T3 cells.** In our previous studies, a reduced level of total and phosphorylated (at Tyr1248) HER2 protein was found in MUC4 down-regulated CD18/HPAF cells (14, 15). Furthermore, we have recently provided evidence that MUC4 interacts with HER2 and regulates its expression (17). In other studies, the rat homologue of MUC4 (rMuc4) has also been shown to interact with ErbB2/HER2/neu and induce its limited phosphorylation (23). Based on these observations, we wanted to determine whether MUC4 regulates the expression of ErbB2 protein and thus of pY1248–ErbB2 in NIH3T3 cells. Our results clearly show that NIH3T3/MUC4 cells have increased levels of total ErbB2 and pY1248–ErbB2 protein compared with the control NIH3T3/pSecTag cells (Fig. 4). MUC4-associated increased expression and activity of HER2 is associated with enhanced phosphorylation of ERK (17). Consistent with our previous observations, NIH3T3/MUC4 cells also showed an increased phosphorylation of ERKs [p42/44 mitogen-activated protein kinase (MAPK)] compared with the control NIH3T3/pSecTag cells.

**MUC4–associated alterations of gene expression in NIH3T3 cells.** DNA oligonucleotide microarrays representing ~10,800 genes were used to identify genes regulated by MUC4 and potentially responsible for tumorigenic potential of MUC4-expressing NIH3T3/MUC4 cells and its effect on the cellular phenotype. The mRNA expression profile of NIH3T3/MUC4 cells was compared with that of NIH3T3/pSecTag cells. Among all the genes, 59 showed at least 1.8-fold change in expression after normalization. Out of these genes, a few selected genes that were either overexpressed or down-regulated in the MUC4-expressing cells are listed in Table 1. Analysis of the data revealed that several growth-associated and energy production–associated genes were up-regulated in the MUC4-expressing cells. Additionally, several genes associated for cell motility were also altered. Genes of particular importance, which were differentially expressed in MUC4-expressing NIH3T3 cells, were those encoding for Nek6, Sna, Gas5, S100A11, Cox3, ND1, Pkp3, HM13, trim16, atf7ip, and Talin. To validate our microarray data, expression of few randomly selected differentially expressed genes was examined by quantitative reverse transcription-PCR (RT-PCR; Supplementary Fig. S1). The results of RT-PCR were in complete agreement with the microarray data, indicating that these alterations in gene expression could be functionally implicated in MUC4-mediated cellular transformation and phenotypic changes of NIH3T3 cells.

**Discussion**

Mucins are high molecular weight glycoproteins secreted by epithelial cells for the lubrication and protection of vulnerable surfaces. Mucins are also believed to play an important role in the pathogenesis of benign and malignant diseases of secretory epithelial cells (24). Our previous studies have shown the specific and differential expression of MUC4 in pancreatic adenocarcinomas compared with the normal pancreas or chronic pancreatitis (6). Furthermore, we have observed 100% incidence of MUC4 overexpression in early stage of ovarian cancer, whereas only a faint staining was observed in some cases of nonneoplastic ovary (25).

An up-regulation of MUC4 has also been observed in variety of other human adenocarcinomas, such as squamous cell carcinoma, lung carcinoma, and mammary and colon cancer (9, 10, 26). *De novo* expression of MUC4 in precancerous pancreatic intraepithelial neoplasias and a progressive increase in its expression with disease advancement implicate MUC4 as an important player in the early and late phases of pancreatic cancer development (20). In fact, our earlier studies have clearly shown the pathogenic functions of MUC4 in pancreatic cancer progression (14, 15). Here, we have presented data showing the oncogenic potential of MUC4 and thus its role in early stages of cancer development. The findings from the present study showed that the ectopic expression of MUC4 increased *in vitro* growth and colony formation in NIH3T3 cells and induced spontaneous tumor formation *in vivo*. MUC4 expression was also associated with increased motility of cells. Moreover, ectopic expression of MUC4 was found to alter the expression of several growth-associated and energy production–associated genes.

Cellular transformation involves the increased expression or activity-promoting mutation(s) in growth-enhancing genes and/or deletion/mutational inactivation of the growth suppressor genes (27). Mucins, MUC4 and MUC1 (another transmembrane mucin), are now well recognized for their growth-promoting activity.

![Figure 4. Effect of MUC4 on ErbB2 and its downstream signaling. Expression of ErbB2, ERK, pY1248–ErbB2, and pERK in NIH3T3 cell sublines was examined by immunoblot analysis. The protein was isolated from subconfluent cultures and 30 μg of protein from each cell extract were resolved by SDS-PAGE (10%), transferred to the PVDF membrane, and probed with antibodies against ErbB2, pY1248–ErbB2, ERK, pERK, and β-actin (internal control). MUC4-transfected NIH3T3 cells showed up-regulated ErbB2, pY1248–ErbB2, and pERK expression compared with empty vector–transfected NIH3T3 cells.](image-url)
were also observed. Sna is a transcription factor that induces regulation of Talin and Pkp3, which are implicated in cell motility, compared with control cells, we did not find any significant increase in mitochondrial proteins is associated with an increase in cancer cells (16). It has been postulated that MUC4-mediated expression of Cox3 and ND1 proteins associated with mitochondrial ATP production. Various NIH3T3 cells up-regulated the expression of Cox3 and ND1 proteins associated with mitochondrial ATP production. Various growth-associated and mitochondrial energy production–associated genes. Furthermore, we also show that expression of MUC4 is associated with enhanced levels of oncprotein ErbB2 and pY1248-ErbB2. An increased phosphorylation of ERK1/2, downstream targets of ErbB2, was also observed. The events downstream of MUC4 that promote tumorigenesis are still unclear but could conclude enhancing conditions for cell proliferation. A model (Fig. 5) has been proposed to depict the possible mechanism of MUC4 in oncogenic transformation. Hence, MUC4 mucin presents an attractive target for cancer prevention and therapeutics with the potential of inhibiting the initiation and growth of human cancer cells.

Table 1. List of selected differentially expressed genes in NIH3T3/MUC4 cells compared with control (NIH3T3/pSecTag) cells

<table>
<thead>
<tr>
<th>Genes up-regulated</th>
<th>Product</th>
<th>Fold change</th>
<th>Function</th>
</tr>
</thead>
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<tr>
<td>Cox3</td>
<td>Cytochrome c oxidase subunit 3</td>
<td>8.969</td>
<td>Oxidative phosphorylation</td>
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<td>ND1</td>
<td>NAD+ dehydrogenase subunit 1</td>
<td>2.485</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td>Nek6</td>
<td>Nima (never in mitosis gene a)–related kinase 6</td>
<td>2.432</td>
<td>Mitotic progression</td>
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<td>trim16</td>
<td>Tripartite motif-containing 16</td>
<td>2.058</td>
<td>Regulation of cell growth</td>
</tr>
<tr>
<td>atf7ip</td>
<td>Activating transcription factor 7 interacting protein</td>
<td>1.922</td>
<td>Regulation of transcription</td>
</tr>
<tr>
<td>IL18R1</td>
<td>Interleukin-18 receptor 1</td>
<td>1.912</td>
<td>Regulation of cell growth</td>
</tr>
<tr>
<td>Sna</td>
<td>Snail homolog 1</td>
<td>1.909</td>
<td>Repression of E-cadherin transcription</td>
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<td>Gas5</td>
<td>Growth arrest–specific 5</td>
<td>1.841</td>
<td>Apoptosis inhibitor</td>
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<tr>
<td>S100A11</td>
<td>S100 calcium-binding protein a11</td>
<td>1.8</td>
<td>Cell growth</td>
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<table>
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<th>Genes down-regulated</th>
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<tr>
<td>Tntr</td>
<td>Talin</td>
<td>0.499</td>
<td>Cell adhesion</td>
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<td>BCAP</td>
<td>B-cell phosphoinositide 3-kinase adaptor</td>
<td>0.489</td>
<td>Regulation of PI3K signaling</td>
</tr>
<tr>
<td>Pkp3</td>
<td>Plakophilin 3</td>
<td>0.471</td>
<td>Cell adhesion</td>
</tr>
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<td>atp8a1</td>
<td>ATPase, aminophospholipid transporter 8a1</td>
<td>0.467</td>
<td>Transport of aminophospholipids</td>
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<td>HM13</td>
<td>Histocompatibility minor 13</td>
<td>0.461</td>
<td>Antigen recognition</td>
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<td>HTR1B</td>
<td>5-Hydroxytryptamine receptor1B</td>
<td>0.427</td>
<td>Neurotransmitter</td>
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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 8/20/2008; revised 9/13/2008; accepted 9/16/2008.

Grant support: U.S. Department of Defense grant OC04110 and NIH grant RO1 CA78590. The University of Nebraska Medical Center Microarray Core Facility receives partial support from NIH grant P20 RR016469 from the IDeA Network of Biomedical Research Excellence Program of the National Center for Research Resources. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Erik Moore for technical support, Dr. Subhankar Chakraborty for help with real-time quantitative PCR, Microarray Core Facility for gene expression analysis, and the Confocal Facility for imaging.

Figure 5. Schematic representation of the proposed mechanism of MUC4 in oncogenic transformation. We propose that MUC4 causes cellular transformation by affecting cellular proliferation (MUC4-ErbB2-Grb2/Sos-Ras-Raf1-MEK-ERK1/2) and cell death (increased expression of mitochondrial energy production genes Cox3 and ND1). In addition, increased expression of growth-promoting genes Nek6 and S100A11 might also be responsible for oncogenic transformation of NIH3T3 cells.

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