Clinical & Experimental Metastasis **21:** 477–483, 2004.
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Review

SGK1, a potential regulator of *c-fms* related breast cancer aggressiveness

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Received 8 May 2004; accepted in revised form 28 September 2004

Key words: breast cancer, *c-fms*, CSF-1, glucocorticoids SGK1

Abstract

The aggressive behavior of breast cancer cells can at times be modulated by hormonal mechanisms. Exposure to glucocorticoids (GC) has been shown to stimulate the invasiveness, motility and adhesiveness of breast cancer cells containing the glucocorticoid receptor. This is largely explained by GC-associated overexpression of the *c-fms* proto-oncogene, which encodes the receptor for the colony stimulating factor-1 (CSF-1). Our objective is to investigate additional GC-associated genetic alterations that could modulate *c-fms* related malignant behavior in breast cancer cells. A microarray technique using an oligonucleotide array representing 16,700 known expressed human genes was used to analyze the gene expression profile of breast cancer cells exposed to dexamethasone (Dex) or vehicle. Results were confirmed by western blot analysis. Six genes were found to be consistently differentially overexpressed in the Dex-exposed cells compared to control. We focused on serum-glucose kinase 1 (SGK1), a serine-threonine kinase known to be involved in intracellular signal transduction pathways and induced by GC and serum. An adhesion assay was performed on extracellular matrix after exposing the breast cancer cells to Dex, CSF-1 or to Dex or CSF-1 plus LY294002, a functional inhibitor of SGK1 action. Exposure to LY294002 significantly decreased both CSF-1 and Dex-induced adhesiveness to the level of control cells. SGK1 may act as a downstream intracellular regulator of *c-fms*, particularly of *c-fms*-induced adhesiveness of breast cancer cells after exposure to GC or CSF-1. This finding may have implications for potential therapeutic interventions aimed at decreasing the aggressiveness of breast cancer cells.

Abbreviations: CSF-1 – colony stimulating factor-1; CSF-1R – colony stimulating factor-1 receptor; GC – glucocorticoids; Dex – dexamethasone; GR – glucocorticoid receptor; EtOH – ethanol; LY – LY294002; SEM – standard error of the mean; PI3-K – phosphatidylinositol 3-kinase

Introduction

Breast cancer is the most common cancer in the females and second most common cause of cancer-related mortality among women in the United States [1]. Breast cancer metastasizes relatively early in the course of the disease. In patients with breast tumor sizes less than 1 cm, the incidence of axillary lymph node involvement is as high as 20% [2], which reflects the aggressiveness of the breast cancer cell.

Recent studies have suggested a role for the growth factor CSF-1[4] and CSF-1R which is encoded by the proto-oncogene *c-fms,* in breast cancer invasiveness. Overexpression of CSF-1R in breast cancer is correlated with increased risk for local relapse [3]. Also, abnormal expression of CSF-1 is associated with a poor outcome in breast cancer patients [4]. Furthermore, transfection of *c-fms* into normal mammary epithelial cells increases cellular invasion and anchorage-independent growth [5].

C-fms can be upregulated in breast cancer cells *in-vitro* by exposure to GC, including Dex, through a GR-dependent pathway [6, 7]. Even physiologic levels of GC markedly increase *c-fms* levels [5]. Exposure of these cells to Dex or CSF-1 also increases invasiveness *in vitro* [5, 8, 9]. Furthermore, we have shown that c-*fms* antisense oligonucleotides delivered into these Dex-stimulated cells interfered with cellular invasiveness, adhesion and motility, suggesting a critical role for *c-fms* in the malignant phenotype of these cells [9]. In addition, in a severe combined immunodeficient mice model of experimental metastasis, we have shown that injecting human breast cancer cells expressing higher levels of *c-fms* into the spleen leads to increased tumorigenicity and intraperitoneal metastasis when compared to control breast cancer cells [10].

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However, the exact mechanism by which GC-related *c-fms* overexpression increases breast cancer cell aggressiveness remains unclear. It is possible that overexpression of *c-fms* after GC stimulation induces further genetic changes downstream that renders the cancer cell more malignant. To our knowledge there are no reports to date that document any genetic changes downstream of *c-fms* after GC stimulation that affect cancer cell aggressiveness.

In this study we sought to investigate additional GC-induced genetic alterations in breast cancer cells that could be associated with increased aggressiveness. Particularly, we were interested in understanding whether GC induces differential expression of genes that could modulate *c-fms* related behavior in breast cancer cells. As a model, we utilized the human breast cancer cell line BT20, which expresses CSF-1R but not its ligand CSF-1, and which is GR positive, but estrogen and progesterone receptor negative [6, 11]. Using a microarray analysis to identify overexpressed genes after exposure to Dex, we found that serum-glucose kinase 1 (SGK1), among other genes, was overexpressed in our model. SGK1 is a gene that encodes a serine/threonine protein kinase and has been found to be involved in cell signaling pathways related to cell survival [12, 13]. We also found that inhibition of SGK1 function by LY significantly decreased breast cancer cell adhesion after exposure to Dex or CSF-1, suggesting a role for SGK1 in the *c-fms* related aggressive phenotype of these cancer cells.

Materials and methods

Cell culture and exposure to different conditions

Human breast cancer cell line BT20 was obtained from American Type Culture Collection (Manassas, VA), and maintained in Eagle's Minimal Essential medium with Earle's BSS and 2 mM L-glutamine supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, California) at 37 °C with 5% CO_2. Cells were starved in serum free media for 48 h before treatment. Forty eight hours before RNA extraction, an EtOH solution of Dex (gift from Dr Richard Hochberg, Yale University), was added at a final concentration of 1.0 μM. EtOH, was used as the control.

RNA preparation, cDNA synthesis, probe labeling, microarray hybridization and analysis

Forty eight hours after exposure to Dex or EtOH, the culture media was decanted, the cells harvested and total RNA was extracted from 80% confluent BT20 cells using Trizol (Invitrogen, Carlsbad, California) following manufacturer's direction. RNA was washed using RNeasy Midi Kit (Qiagen, Valencia, California) and further concentrated using microcon YM-30 columns (Millipore, Billerica, Massachusetts) to about 3.3–6.6 μg/μl in DEPC water. Approximately 50 μg of total RNA per condition was used to synthesize cDNA. cDNA probes were synthesized by reverse transcription using oligodeoxythymidine and amino-allyl dUTP (Sigma, St Louis, Missouri) at a final concentration of 1 μg/μl and 200 μM, respectively. A labeling mix for the reverse transcription was prepared by adding the following: dATP, dCTP, dGTP to final concentration of 500 μM each, dTTP to a final concentration of 200 μM, and 2 μl Superscript-RT (Invitrogen). The mix was first incubated for 90 min at 42 °C before 2 μl of Superscript-RT was added again for another 2-h incubation. RNA was then eliminated by adding to each tube 5 μl of 0.5 M EDTA and 10 μl of 1 M NaOH, and heating for 20min at 60–65 °C. NaOH was neutralized with 6 μl 1 M HCl, and 1 M Tris pH 7.5. cDNA probe cleanup was accomplished by performing a phenol–chloroform extraction. Probes were concentrated on a microcon YM-30 column (13,000 rpm centrifugation for 7 min). This process was repeated three times. Probes were labeled with 15 μg of cyanine-3 or cyanine-5 mono-reactive dyes (Amersham, Piscataway, New Jersey) incubated in the dark, at room temperature, for 90 min after which 1 μl of 2 M ethanolamine (Sigma) was added. Probes were then purified on microcon YM-30 columns as described above. Denaturation and prehybridization of probes was performed by mixing the labeled cDNA with 5 μg/μl of poly dA (Amersham), 3.2 μl of 20 × SSC and 2.5 μl 2% SDS. The mix was then heated at 90 °C for 2 min.

Microarray slides containing 16,659 70-mer oligonucleotides representing expressed human genes were obtained from the Keck Microarray Laboratory, Yale University. The pre-hybridized mix containing the probes was then applied on each slide containing the arrays and the slides were secured in a HybChamber (GeneMachines, San Carlos, California). Hybridization buffer consisting of 3.2 × SSC and 23% formamide was added to the chambers. The hybridization chambers containing the microarray slides were incubated overnight at 55° C. The array slides were washed the next day by using alternating solutions of 1 × SSC/0.03% SDS, 0.2 × SSC and 0.05 × SSC.

After drying, the slides were scanned on an Axon 4000B laser scanner (Axon Instruments, Union City, CA), with laser power adjusted so that the distribution of pixel intensities for each of the two wavelengths was balanced, i.e. was close to 1.0. Dual-color images were analyzed using the GenePix Pro software version 3.0 (Axon Instruments). Regions with high background, irregular spots, missing spots and spots with fluorescence lower than the background were flagged for elimination from further analysis.

The experiment described above was repeated independently four times using identical conditions. All result files were imported into the GeneSpring 4.2 software (Silicon Genetics, Redwood City, California)

and simultaneously analyzed for gene expression differences. Data was normalized using per-spot and per-chip intensity-dependent (Lowess) normalization. Our analysis was focused on genes with an arbitrary cut-off value of > 2.5-fold change in their Cy5 over Cy3 value for overexpressed genes, or < 0.5-fold for genes that were underexpressed. Data was filtered both at the expression level, to remove genes with median fluorescence lower than the standard deviation of the background, and at the level of confidence, using per gene, one sample Student's *t*-test *P*-values calculated by GeneSpring for replicate data. An arbitrary *P*-value of ≤ 0.01 was used to select genes with the highest chance of differential expression.

Western blot analysis

BT20 cells were exposed to Dex or EtOH as described above. After 48 h exposure, cells were harvested and total protein extract was prepared by incubating the cell pellets in gentle lysis buffer [25 mM HEPES, pH 7.9, 150 mM KCl, 1 mM EDTA, 10 mM Na fluoride, 0.1% NP-40, 1 mM DTT, and 1X protease inhibitor (Calbiochem, San Diego, California)], followed by centrifugation for 5 min at 14,000 rpm in a microcentrifuge. One hundred microgram of protein per condition was resolved on a 10% SDS-PAGE and transferred to 0.45 μM PVDF membranes (Millipore). The membranes were first blocked in 1X phosphate-buffered saline with 5% skim milk and 0.1% Tween-20 for 1 h at room temperature. The membranes were then probed with a 1 : 1000 dilution of anti-SGK1 rabbit polyclonal antibody (kind gift of Dr Florian Lang, Tuebingen, Germany) for 1 h at room temperature in blocking buffer. Horseradish peroxidase-labeled anti-rabbit IgG, (Promega, Madison, Wisconsin) at a dilution of 1/10,000 for 1 h at room temperature was used secondary antibody. The same blot was probed with a 1/1000 dilution of anti-FKBP51 goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California), followed by incubation with a 1/10,000 dilution of horseradish peroxidase-labeled anti-goat IgG secondary antibody (Santa Cruz Biotechnology). An antibody against GAPDH (Santa Cruz Biotechnology) was also used at 1/30,000 dilution for sample loading control. The blots were further developed using western blot chemiluminescence reagent (NEN, Boston, Massachusetts) and the intensity quantified using Adobe Photoshop software version 6.0.

Adhesion assay

BT20 cells were cultured as described above. Cells were first starved for 24 h followed by treatment conditions for 24 more hours. The cells were exposed to the following conditions while in starvation media: Dex at 1 μM plus LY (Calbiochem) at 50 μM, Dex alone, LY plus EtOH, CSF-1 at 250 ng/ml plus LY, CSF-1 alone, and ETOH alone. After 24-h of exposure, the cells were harvested and an adhesion assay was performed as described previously [14]. Briefly, each group of cells representing the different conditions were washed with PBS and re-suspended in starvation media plus 1% Nu-Serum (BD Biosciences). A 10-μM pore polycarbonated filter (Corning, Corning, New York) was coated with human defined matrix containing 50 μg/ml human laminin, 50 μg/ml human collagen IV and 2 mg/ml gelatin in 10 mM acetic acid. A Membrane Invasion Culture System chamber was covered with the matrix-coated filter. 1×10^5 cells were placed in each well and then incubated at 37 °C for 2 h. The filter was then fixed in 0.5% crystal violet in 25% methanol. Adherent cells in each condition were counted under 40 × lens and percent adhesion ± SEM was calculated.

Results

Differential gene overexpression after stimulation with Dex

To examine the differential expression of genes after exposure of the human BT20 breast cancer cells to Dex, a microarray technique using a slide with 16,659 70-mer oligonucleotides representing known expressed human genes, was utilized. Analysis and quantification of the gene expression profile of the combination of all four experiments revealed six genes that showed consistent overexpression using an arbitrary cut-off of 2.5-fold change in their Cy5 over Cy3 and a $P \leq 0.01$. In addition, seven genes showed consistent downregulation using an arbitrary cut-off of 0.5-fold and a $P \leq 0.01$ (Table 1).

The six overexpressed genes identified are: (a) SGK1, which encodes a 50-kDa serine/threonine kinase that is known to be induced by GC [15, 16]. Moreover, SGK1 has also recently been found to be involved in cell signaling pathways related to cell survival [12, 13]. SGK1 has been shown to be involved in GC-induced cell survival [17]; (b) FKBP51, which encodes immunophilin that acts as the receptor for the immunosuppressant FK506 (Tacrolimus) and is known to be part of the GC and progesterone receptor complexes [18]; (c) MAO-1 that encodes the enzyme monoamine oxidase A which catalyzes the oxidative deamination of biogenic and xenobiotic amines and has important functions in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues [19]; (d) GW112, which is a gene recently cloned from human myeloblasts and is found to be selectively expressed in inflamed colonic epithelium. Its exact function is not yet known [20]; (e) 11-B-DH2, which encodes an enzyme responsible for glucocorticoid metabolism [21] and (f) PER1, which encodes a circadian regulator that may act as a transcription factor and may be a pacemaker component which responds to light and mediates photic entrainment in the retina [22].

Table 1. Mean and range of expression level of selected genes after exposure to dexamethasone.

Genes	Mean expression	Expression range	*P*
FKBP51	5.8	2.7–10.6	0.006
GW112	3.7	2.0–5.7	0.006
MAO-1	3.6	2.5–5.1	0.003
PER1	2.7	2.2–3.0	0.007
SGK1	2.5	1.9–3.4	0.004
11-B-DH2	2.5	1.9–3.0	0.001
HUMGT198A	0.4	0.4–0.4	0.01
ENC1	0.5	0.3–0.8	0.01
B7-H4	0.5	0.3–0.8	0.01
UGT1A10	0.5	0.4–0.6	0.001
STAC	0.5	0.5–0.6	0.001
BAMB1	0.5	0.4–0.6	0.002
PPAP2B	0.5	0.4–0.7	0.003

Human breast cancer cells were exposed to dexamethasone (Dex) for 48 hrs. A microarray technique using an oligonucleotide array representing 16,700 known expressed human genes was used to analyze the gene expression profile of cells exposed to Dex or vehicle.
The slides were scanned on an Axon 4000B laser scanner. Analysis and quantification of the fluorescence hybridization signal of the microarray slide was performed using the GenePixPro software version 3.0. The result of the analysis from four exact repetitions of the experiment were combined and simultaneously analyzed using the GeneSpring software version 4.2 for gene expression differences. Data were normalized using per-spot and per-chip intensity dependent (Lowess) normalization. Our analysis was focused on genes with an arbitrary cutoff value of >2.5 fold change in their Cy5 over Cy3 value for overexpressed genes, or <0.5-fold for genes that were underexpressed. A Student's *t*-test was calculated by GeneSpring for replicate data. An arbitrary *P*-value of ≤ 0.01 was used to select genes with the highest chance of differential expression.

In summary, using gene expression microarray analysis, we identified six genes that are consistently overexpressed and seven genes that are downregulated in human breast cancer cells after exposure to GC.

Confirmation of gene overexpression by western blot analysis

To verify the level of overexpression of genes identified by microarray, protein expression of these genes after Dex stimulation was analyzed. Of these six genes, we focused our investigations on SGK1 due to its known role in cell signaling pathways that might be involved in the invasiveness of the cancer cell. Using western blot analysis and an anti-SGK1 polyclonal antibody raised against SGK1 of human origin, we confirmed that SGK1 is indeed overexpressed 3-fold when BT20 cells were exposed to Dex compared to control. We also confirmed overexpression of the 51 kDa FKBP51 protein. For this particular antibody, two bands were identified in the group exposed to Dex, the upper band likely representing the phosphorylated form. This band is overexpressed by 5-fold and the lower band by 3-fold when compared to non-treated cells (Figure 1). These western blot findings validate our microarray analysis.

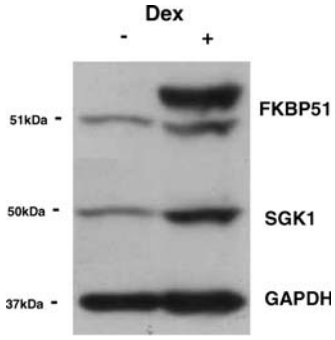

Figure 1. Confirmation of upregulation of SGK1 and FKBP51 after exposure to Dex. BT20 cells were treated with Dex (1.0 μM) or vehicle (control) for 48 h. Total protein extracts were subjected to 10% SDS-PAGE, followed by immunobloting with SGK1 and FKBP51 antibodies. GAPDH was used as the loading control. After exposure to Dex, SGK1 is overexpressed 3-fold and FKBP51 is overexpressed by 5-fold when compared to control.

Potential role of SGK1 in breast cancer cell adhesiveness induced by Dex or CSF-1

We have shown previously that Dex augments invasion, motility and adhesion of BT20 cells whereas *c-fms* antisense oligonucleotide delivered into these Dex stimulated cells interfered with these malignant properties, suggesting a critical role for *c-fms* in the malignant phenotype of these cells [9]. Specifically, antisense *c-fms* decreased adhesiveness of Dex-stimulated BT20 cells by approximately 3-fold, compared to control scrambled oligomers.

We then set to find out the role of SGK1 in this *c-fms*-related breast cancer model, since we have shown SGK1 to be significantly overexpressed after exposure to Dex. To address this question, we investigated whether blocking SGK1 function had any effect on Dex or CSF-1-induced adhesiveness of BT20 cells to human extracellular matrix, as a surrogate for cellular malignant behavior. Previous studies have shown SGK1 to be regulated at several levels including (a) at the transcriptional level by up-regulation of mRNA production after exposure to Dex, serum or cellular stress [17]; (b) by reversible phosphorylation as a direct downstream target of PI3-K pathway [12, 22]; and (c) by the ubiquitin–proteasome pathway [23]. Therefore, we were interested in blocking SGK1 function. Since there are no known direct SGK1 inhibitors, we chose to block SGK1 function by using LY, a specific inhibitor of PI3-kinase, which is upstream of SGK1 and has been shown to cause inhibition of SGK1 function by inhibiting SGK1 phosphorylation [24, 25].

We first tested the effect of LY on Dex-stimulated adhesiveness, since Dex-stimulates both *c-fms* and SGK1 expression. Our results confirmed that Dex increases adhesiveness of BT20 cells. Dex increased adhesiveness was effectively inhibited almost 2-fold by the addition of LY, from 50.9 $\pm$ 2.1 to 27 $\pm$ 3.0, similar to the level of adhesiveness seen after exposure to LY alone without any Dex stimulation (31.2 $\pm$ 1.9)

(Figure 2). There was no effect of LY on adhesiveness of BT20 cells compared to control cells exposed to EtOH.

Next, we explored the effect of LY on CSF-1-stimulated adhesiveness which correlates with *c-fms* function specifically. Exposure of the BT20 cells to CSF-1 led to a similar level of adhesiveness as did Dex. Moreover, we found that CSF-1-induced adhesiveness was also inhibited by LY, from 62.1 ± 5.8 to 30.3 ± 3.6, down to the level of adhesiveness seen after exposure of cells to LY alone without CSF-1-stimulation. In summary, we showed that indirect inhibition of SGK by LY inhibited both GC and CSF-1-related adhesiveness in breast cancer cells. These results combined suggest a critical role for SGK1 in both the Dex and CSF-1-induced adhesiveness of the breast cancer cell through the *c-fms* pathway.

Discussion

We have confirmed that SGK1 is overexpressed in human breast cancer cells after exposure to GC. Furthermore, we have demonstrated that treatment with LY, which inhibits phosphorylation of SGK1 significantly decreases breast cancer cell adhesiveness, suggesting a novel pathway of *c-fms*-related aggressiveness in human breast cancer cells through over-expression of SGK1.

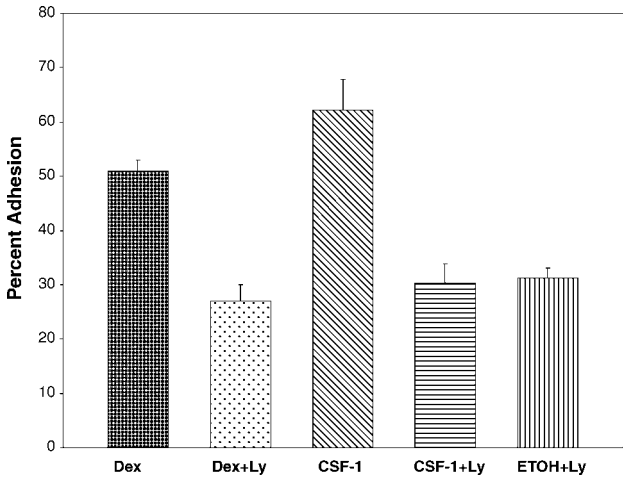

Figure 2. Adhesiveness of BT20 cells. BT20 cells were placed in starvation media for 24 h and exposed for 24 h to the following conditions while in starvation media: Dex (1 μM) plus LY (50 μM), Dex alone, LY plus EtOH, CSF-1 (250 ng/ml) plus LY, and CSF-1 alone. Cells were then re-suspended in media plus 1% NuSerum. A film of 10 μM pore polycarbonated filter was coated with human defined matrix containing 50 μg/ml human laminin, 50 μg/ml human collagen IV and 2 mg/ml gelatin in 10 mM acetic acid. A Membrane Invasion Culture System chamber was covered with the matrix-coated filter. 1×10^5 cells were placed on top of the filter and then incubated at 37 °C for 2 h. The filter was then fixed in 0.5% crystal violet in 25% methanol. Adherent cells in each condition were counted under 40× lens and percent adhesion was calculated. Error bars indicate SEM

It is well established that SGK1 is upregulated at the transcriptional level after stimulation with GC in different human cell types [16, 17]. It has also been shown that SGK1 activity increases after its phosphorylation, which is PI3-K-dependent [22]. More recently, Mikosz et al. described SGK1 up-regulation by GC in human breast cancer cell lines, including BT20 [17]. Our report confirmed these results using a gene expression microarray as well as western blot analysis.

GC upregulation of the *c-fms* proto-oncogene encoding CSF-1R has been well established in human breast cancer cells [6, 7] as well as in choriocarcinomas[26]. After stimulation of *c-fms* by GC, breast cancer cells increased invasiveness, motility and adhesiveness, whereas incubation of these GC-stimulated cells with antisense oligonucleotides targeted against *c-fms* significantly interfered with these phenotypes, demonstrating the importance of the autocrine loop between *c-fms* and CSF-1 in breast cancer cell aggressive behavior [9].

The phenotypic effect of SGK1 after GC stimulation in malignant cells has not been thoroughly investigated previously. GC-induced SGK1 upregulation has been associated with a cell survival effect by inhibition of apoptosis [27] in breast cancer cells [17], which adds to its potential role as a stimulator of malignant behavior. The invasiveness of cancer cells depends in part on the ability of the cell to adhere. This is a phenotype that contributes to aggressiveness of malignant cells and can be measured *in vitro* [14]. In this study, using cellular adhesiveness as surrogate for malignant cell aggressive behavior, we showed that LY, which has been shown to inhibit SGK1 phosphorylation, decreased GC-related cellular adhesiveness.

Previous work has established a potential relationship between *c-fms,* PI3-K and SGK1. Stimulation of *c-fms* with its ligand CSF-1 induces activation of PI3-K, which is directly upstream from SGK1 and causes its phosphorylation [28–30]. One would expect that upregulation of *c-fms* by GC may also result in the same effect, although this has not been directly demonstrated to date. In other models, GC increased the abundance of SGK1 protein in a PI3-K independent manner. By using the PI3-K specific inhibitor LY, which inhibits the phosphorylation of SGK1 [25], we showed that LY blocked cellular adhesiveness after CSF-1 exposure, suggesting that SGK1 acts downstream of *c-fms* activation by CSF-1.

Our studies of effect of LY on Dex-stimulated breast cancer cell adhesiveness further suggests that GC adds to this pathway by upregulating SGK1 at the transcriptional level (as demonstrated in the microarray assay). By increasing *c-fms* expression, Dex may also stimulate SGK1 functional effect through a *c-fms*-PI3-kinase dependent pathway, another level of control of *c-fms*/SGK1 related function.

Since LY has been shown to inhibit PI3-K directly and SGK1 only indirectly, an alternative hypothesis

could be that LY-related decreased cellular adhesiveness after GC exposure may be due to inhibition of other kinases downstream from PI3-K along with SGK1. In addition, CSF-1 has been shown to activate the PI3-K pathway and phosphorylate AKT, a kinase with a strong homology to SGK1 and is also involved in cell survival pathways [29, 31]. A recent report by Brunet et al. demonstrated that PI3-K-activated kinases SGK1 and AKT played a significant role in mediating the cell survival signal [13]. It is possible that LY inhibition of AKT and/or other PI3-K activated kinases, and not only SGK, are responsible for the decreased adhesiveness. Although in our microarray analysis AKT was not overexpressed after GC exposure, it is feasible that increased AKT activity might not translate into expression. Survival signals mediated by PI3-K activity seem to be fairly specific. In Brunet's paper, other kinases also known to be activated by PI3-K phosphorylation besides SGK1 and AKT, were not involved in cell survival signaling. Moreover, Leong et al. have recently demonstrated that in mammary epithelial cells exposed to GC, SGK1 but not AKT was phosphorylated through PI3-K, concluding that although SGK1 and AKT share upstream activators, their responses are different and probably regulated in a cell type and tissue-specific manner. The effect of LY on other genes that might be involved in decreased cellular adhesiveness after GC exposure warrants further investigation.

In summary, we have identified an important downstream modulator of *c-fms* malignant cellular aggressiveness in breast cancer cells by which stimulation with GC of human breast cancer cells may cause SGK1 overexpression and increased cellular adhesion, and treatment with LY, which inhibits SGK1 phosphorylation, any block both GC and CSF-1-induced adhesiveness. This finding may have implications for potential therapeutic interventions aimed at decreasing the aggressiveness of breast cancer cells.

Acknowledgement

This work was supported by Department of Defense grant DAMD 17-02-1-0633 to S.K. Chambers.

References

1. Jemal A, Murray T, Samuels A et al. Cancer statistics, 2003. CA Cancer J Clin 2003; 53: 5–26.
2. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989; 63: 181–7.
3. Maher MG, Sapi E, Turner B et al. Prognostic significance of colony-stimulating factor receptor expression in ipsilateral breast cancer recurrence. Clin Cancer Res 1998; 4: 1851–6.
4. Scholl SM, Pallud C, Beuvon F et al. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. J Natl Cancer Inst 1994; 86: 120-6.
5. Sapi E, Flick MB, Rodov S et al. Independent regulation of invasion and anchorage-independent growth by different autophosphorylation sites of the macrophage colony-stimulating factor 1 receptor. Cancer Res 1996; 56: 5704-12.
6. Chambers SK, Wang Y, Gilmore-Hebert M et al. Post-transcriptional regulation of *c-fms* proto-oncogene expression by dexamethasone and of CSF-1 in human breast carcinomas *in vitro*. Steroids 1994; 59: 514-22.
7. Sapi E, Flick MB, Gilmore-Hebert M et al. Transcriptional regulation of the *c-fms* (CSF-1R) proto-oncogene in human breast carcinoma cells by glucocorticoids. Oncogene 1995; 10: 529-42.
8. Filderman AE, Bruckner A, Kacinski BM et al. Macrophage colony-stimulating factor (CSF-1) enhances invasiveness in CSF-1 receptor-positive carcinoma cell lines. Cancer Res 1992; 52: 3661-6.
9. Azodi M, Roy WJ, Chambers JT et al. Inhibition of invasiveness and motility of ovarian and breast cancer cells by oligomer therapy directed towards the *c-fms* proto-oncogene. Proc Int Gynecol Cancer Soc 1999.
10. Toy EP, Bonafe N, Savlu A et al. Correlation of tumor phenotype with c-*fms* proto-oncogene expression in an *in vivo* intraperitoneal model for experimental human breast cancer metastasis. Clin Exp Metast, submitted.
11. Sapi E, Flick MB, Rodov S et al. Ets-2 transdominant mutant abolishes anchorage-independent growth and macrophage colony-stimulating factor-stimulated invasion by BT20 breast carcinoma cells. Cancer Res 1998; 58: 1027–33.
12. Park J, Leong ML, Buse P et al. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. EMBO J 1999; 18: 3024–33.
13. Brunet A, Park J, Tran H et al. Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol 2001; 21: 952–65.
14. Chambers SK. *In vitro* invasion assays. In Bartlett JMS (ed): Methods in Molecular Medicine. Totowa, New Jersey: Humana Press 2000; 179–86.
15. Webster MK, Goya L, Firestone GL. Immediate-early transcriptional regulation and rapid mRNA turnover of a putative serine/threonine protein kinase. J Biol Chem 1993; 268: 11482–5.
16. Webster MK, Goya L, Ge Y et al. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol Cell Biol 1993; 13: 2031–40.
17. Mikosz CA, Brickley DR, Sharkey MS et al. Glucocorticoid receptor-mediated protection from apoptosis is associated with induction of the serine/threonine survival kinase gene, sgk-1. J Biol Chem 2001; 276: 16649–54.
18. Pirkl F, Buchner J. Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. J Mol Biol 2001; 308: 795–806.
19. Hsu YP, Weyler W, Chen S et al. Structural features of human monoamine oxidase A elucidated from cDNA and peptide sequences. J Neurochem 1988; 51: 1321–4.
20. Shinozaki S, Nakamura T, Iimura M et al. Upregulation of Reg 1alpha and GW112 in the epithelium of inflamed colonic mucosa. Gut 2001; 48: 623–9.
21. Agarwal AK, Rogerson FM, Mune T et al. Gene structure and chromosomal localization of the human HSD11K gene encoding the kidney (type 2) isozyme of 11 beta-hydroxysteroid dehydrogenase. Genomics 1995; 29: 195–9.
22. Kobayashi T, Deak M, Morrice N et al. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem J 1999; 344 Pt 1: 189–97.
23. Brickley DR, Mikosz CA, Hagan CR et al. Ubiquitin modification of serum and glucocorticoid-induced protein kinase-1 (SGK-1). J Biol Chem 2002; 277: 43064–70.
24. Buse P, Tran SH, Luther E et al. Cell cycle and hormonal control of nuclear-cytoplasmic localization of the serum- and glucocorticoid-inducible protein kinase, Sgk, in mammary tumor cells. A novel convergence point of anti-proliferative and proliferative cell signaling pathways. J Biol Chem 1999; 274: 7253–63.

25. Wang J, Barbry P, Maiyar AC et al. SGK integrates insulin and mineralocorticoid regulation of epithelial sodium transport. Am J Physiol Renal Physiol 2001; 280: F303–13.
26. Chambers SK, Ivins CM, Kacinski BM et al. An unexpected effect of glucocorticoids on stimulation of *c-fms* proto-oncogene expression in choriocarcinoma cells expressing little glucocorticoid receptor. Am J Obstet Gynecol 2004; 190: 974–85.
27. Leong ML, Maiyar AC, Kim B et al. Expression of the serum- and glucocorticoid-inducible protein kinase, Sgk, is a cell survival response to multiple types of environmental stress stimuli in mammary epithelial cells. J Biol Chem 2003; 278: 5871–82.
28. Bourette RP, Myles GM, Choi JL et al. Sequential activation of phoshatidylinositol 3-kinase and phospholipase C-gamma2 by the M-CSF receptor is necessary for differentiation signaling. EMBO J 1997; 16: 5880–93.
29. Kelley TW, Graham MM, Doseff AI et al. Macrophage colony-stimulating factor promotes cell survival through Akt/protein kinase B. J Biol Chem 1999; 274: 26393–8.
30. Grey A, Chen Y, Paliwal I et al. Evidence for a functional association between phosphatidylinositol 3-kinase and c-src in the spreading response of osteoclasts to colony-stimulating factor-1. Endocrinol 2000; 141: 2129–38.
31. Lee AW, States DJ. Both src-dependent and -independent mechanisms mediate phosphatidylinositol 3-kinase regulation of colony-stimulating factor 1-activated mitogen-activated protein kinases in myeloid progenitors. Mol Cell Biol 2000; 20: 6779–98.