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14. ABSTRACT

My experiments indicate that Serum and Glucocorticoid regulated Kinase (SGK) proteins facilitate breast cancer invasive migration, a critical step for ultimate cancer metastasis to other organs. The activation of SGK during cellular stress conditions, such as low oxygen found within a tumor, makes this data increasingly imperative for therapeutics. The research shown here demonstrates SGK loss in highly mestatic breast cancer cell lines causes an invasive migration defect. Conversely, the overexpression of SGK isoforms in breast cancer cell lines causes an enhancement of invasive migration. These discoveries are helping to elucidate new mechanisms that can be targeted for more specific and successful therapies to block breast cancer metastasis. Known targets of SGK proteins are being examined for their contribution to the metastatic properties of breast cancer cells. These studies will determine the importance of SGK proteins as putative therapeutic targets for breast cancer motility.

15. SUBJECT TERMS

Serum and Glucocorticoid Regulated Kinase (SGK), breast cancer cell migration and invasion, retroviral overexpression, lentiviral silencing

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Introduction

Breast cancer arises as a result of alterations in the cell signaling networks that control growth, division, and metastasis. Knowledge of the details of these signaling systems is becoming increasingly essential for the design of new and effective treatments. One such pathway is phosphoinositide 3-kinase (PI3K) which activates numerous downstream effectors, which in turn regulate a plethora of physiological and pathophysiological process. In human cancer, two critical PI3K effectors are the protein kinases Akt, the human homologue of the viral oncogene *v-Akt,* and SGK, the Serum and Glucocorticoid-regulated Kinase. The importance of this pathway in disease is underscored by the fact that the gene encoding the catalytic subunit of PI3K, *PIK3CA*, is one of the most frequently mutated oncogenes in breast cancer. The two most common *PIK3CA* somatic oncogenic mutations occurring in breast cancer are E542K, in the helical domain, and H1047R, in the kinase domain, and both mutations result in hyperactive PI3K signaling (1). The oncogenic *PIK3CA* mutations have been documented at a frequency 25-40% in breast tumors (2,3). Therefore, evaluating the function of downstream effectors of PI3K in breast cancer, such as Akt, has been a cancer biology focus for some time. Numerous studies have revealed PI3K and Akt as a central signaling node integrating processes of cell growth, survival, and proliferation. Recently our laboratory discovered that distinct isoforms of Akt (Akt1 and 2) also play a key role in breast cancer cell invasive migration, whereby Akt1 inhibits and Akt2 enhances invasive migration (4). Despite the wealth of knowledge concerning PI3K and Akt signaling, relatively little is known about SGK, also a PI3K effector. SGK isoforms share many characteristics with Akt, such as high homology in the catalytic domain, common mechanism of phosphorylation and activation, a significant number of overlapping substrates, and deregulation in breast carcinoma (5,6). To date, no studies have investigated any role for SGK in cell invasive migration or metastasis. This is despite the fact that SGK is also activated by oncogenic PI3K and that SGK isoforms are documented as amplified with high frequency in breast cancer(6). This makes the similarities and distinctions between Akt and SGK attractive targets for therapeutic applications in cancer.

Body

The following tasks from the Statement of Work for this project were the focus for the research period from March 15, 2010- March 14, 2011: Examine the influence of SGK isoforms on cellular motility both individually and in concert with Akt isoforms (months 1-19)

1.1 Optimize plasmids and transfection techniques to induce overexpression of Akt and SGK isoforms in breast carcinoma cell lines (month 1)

1.2 Optimize lentiviral infection technique and siRNA silencing sequences for Akt and SGK isoforms in breast carcinoma cell lines (months 2-3)

1.3 Optimize lentiviral infection for PIK3CA and refractory PIK3CA overexpression in MCF10A breast cells (month 4-7)

1.4 Overexpress SGK and Akt isoforms individually in normally migratory breast cancer cell lines and normally static breast cell lines and evaluate motility changes via Transwell migration and Transwell Matrigel invasion assays (months 8-10)

1.5 Overexpress SGK isoforms in concert with Akt isoforms and evaluate motility changes with Transwell migration and Transwell Matrigel invasion assays in normally migratory breast cancer cell lines and normally static breast cell lines (months 11-13)

Progress

1.1 Optimize plasmids and transfection techniques to induce overexpression of Akt and SGK isoforms in breast carcinoma cell lines (month 1)

In order to accurately compare the significance of Akt and SGK isoforms in breast cancer we optimized their transfection to produce robust and equivalent expression giving reproducible outcomes in a breast cancer cell system. SUM159PT breast cancer cells were used to initially test equivalence of plasmid expression **(FIGURE 1A)** and HS578T cells were use to show titration of SGK1 in breast cancer cells to combat issues with their degradation and instability **(FIGURE 1B)**.

1.2 Optimize lentiviral infection technique and siRNA silencing sequences for Akt and SGK isoforms in breast carcinoma cell lines (months 2-3)

To insure specificity of SGK knockdown in breast cancer cell lines I made two different specific pLKO shRNAs for SGK1 and two specific pLKO shRNAs for SGK3 and tested them in breast cancer cells known to have significant endogenous expression of the isoforms **(FIGURE 2)**. Initially RT-PCR was done in a number of common breast cancer cell lines to determine significance of SGK isoform expression in breast cancer. SGK1 and SGK3 were both expressed in a number of breast cancer cell lines, while SGK2 expression was not **(FIGURE 3)**. This experiment agrees and validates

tissue data from a number of labs showing that SGK2 is a tissue specific kinase that is not expressed in normal mammary tissue. With this data we will proceed focusing upon SGK1 and SGK3, which were shown by our lab and others to be expressed and often overexpressed in breast cancer.

1.3 Optimize lentiviral infection for PIK3CA and refractory PIK3CA overexpression in MCF10A breast cells (month 4-7)

I tested five different PIK3CA/pLKO shRNA constructs to determine the shRNA with the most efficient silencing of the PIK3CA protein, p110α **(FIGURE 4)**. The overexpression of PIK3CA in MCF10A cells was attempted multiple times in multiple ways, but the efficiency of the retrovirus was insufficient to express quantitative amounts of p110α. In its stead, transfection overexpression vectors were used for PIK3CA and its two most common breast cancer mutation hotspots, E545K and H1047R. Upon transfection of E545K and H1047R PIK3CA mutations in 293T cells a significant increase in phosphorylation of SGK3 at Ser486 was demonstrated, while the transfection of wildtype PIK3CA produced no increase in the phosphorylation and thus activity of SGK3 **(FIGURE 5).**

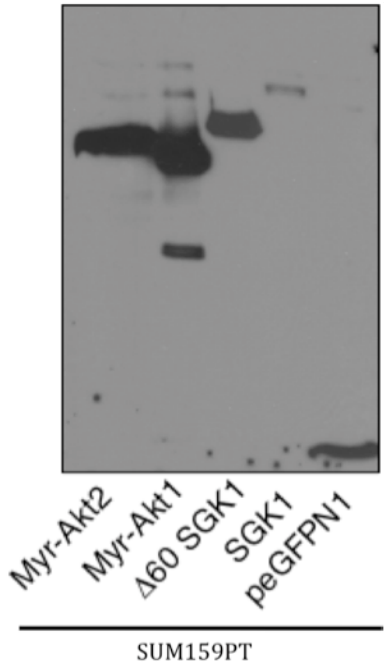
1.4 Overexpress SGK and Akt isoforms individually in normally migratory breast cancer cell lines and normally static breast cell lines and evaluate motility changes via Transwell migration and Transwell Matrigel invasion assays (months 8-10)

All three SGK isoforms significantly enhance breast cancer cell migration in SUM159PT **(FIGURE 6A).** SGK1 and SGK3 overexpression also enhance breast cancer cell migration in HS578T cells and concordantly enhanced breast cancer invasion in HS578T breast cancer cell invasion in Transwell Matrigel invasion assays **(FIGURE 6B**). Additionally morphogenic changes were seen in the HS578T breast cancer cells up on overexpression of SGK isoforms. SGK causes the cells to become spread out and globular as opposed to control treated cells, while SGK3 causes the cells to become more elongated spindle like in shape upon overexpression compared to control treated cells **(FIGURE 7).**

1.5 Overexpress SGK isoforms in concert with Akt isoforms and evaluate motility changes with Transwell migration and Transwell Matrigel invasion assays in normally migratory breast cancer cell lines and normally static breast cell lines (months 11-13)

Initial attempts did not yield equivalent expression of Akt and SGKs when dually transfected.

Figure 1A



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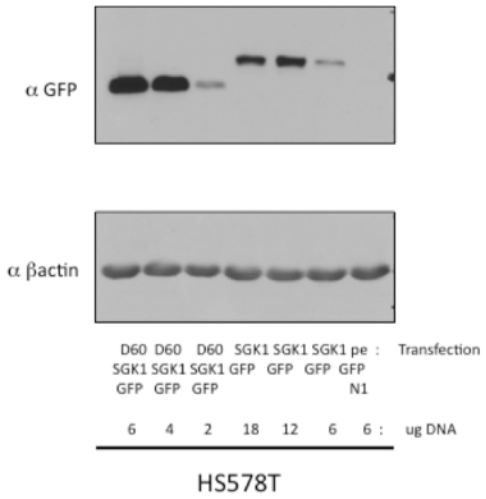

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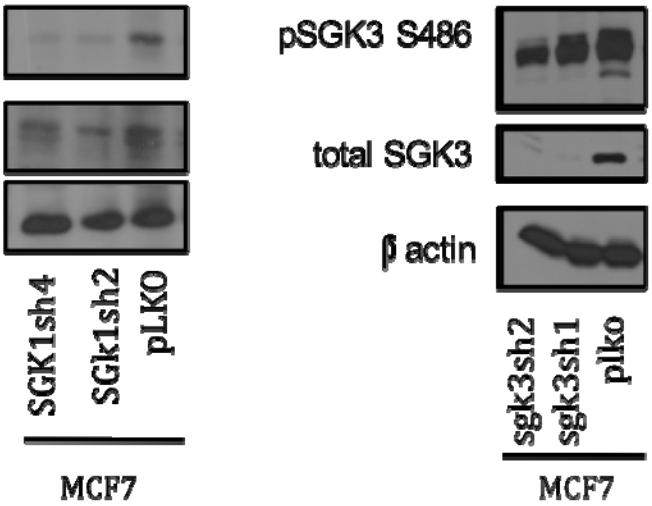

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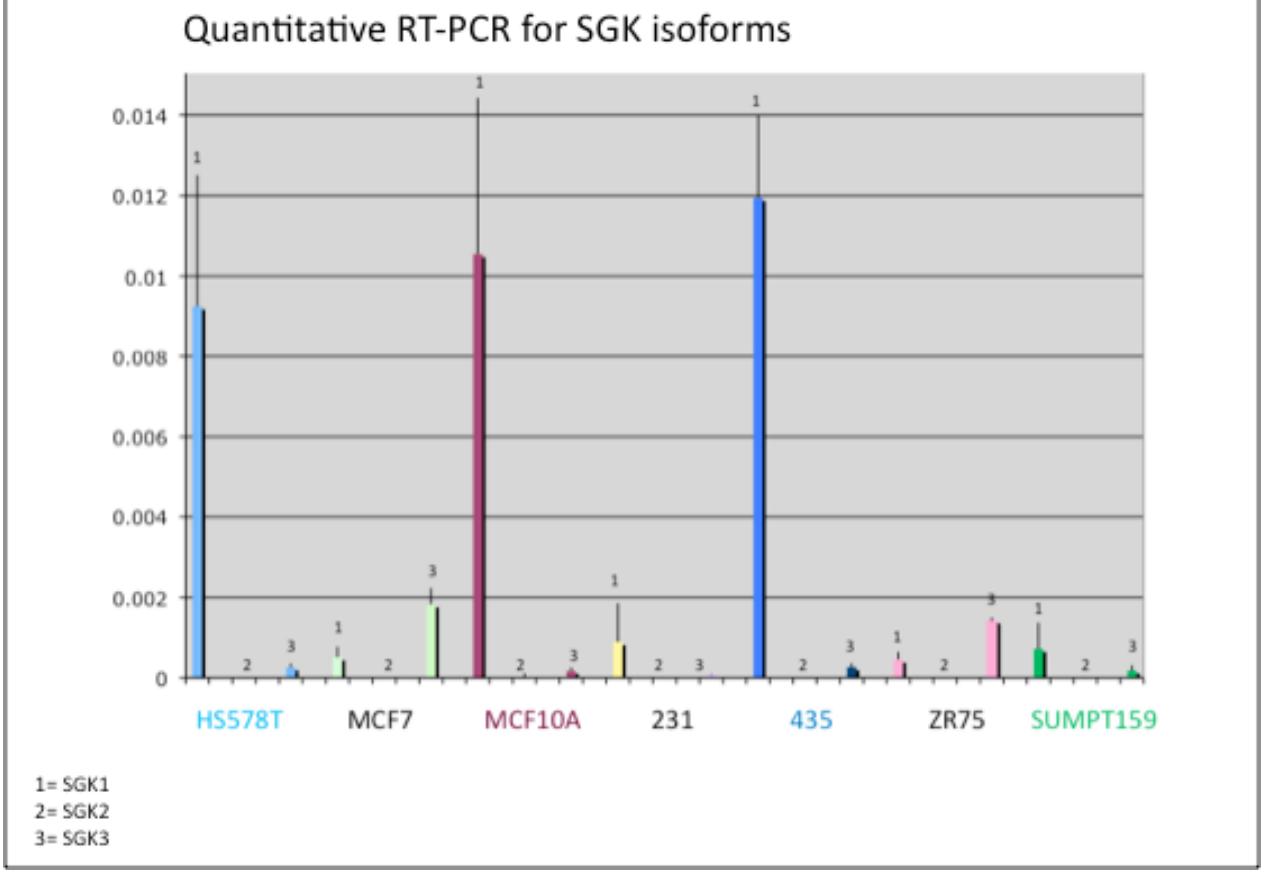

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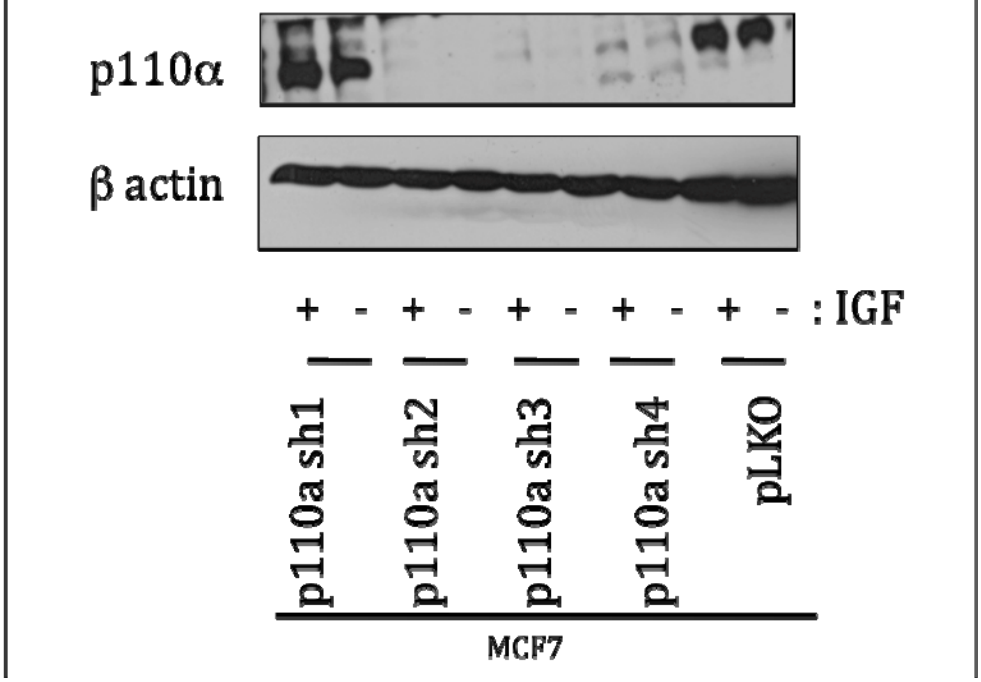


Current experimental efforts have focused upon examining the concordant expression of Akt and

Figure 5

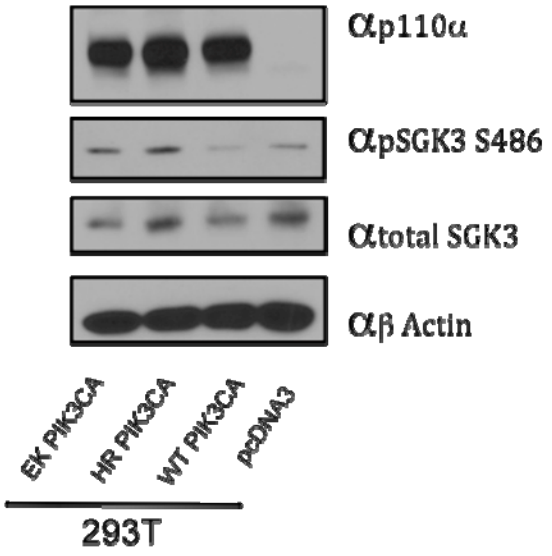
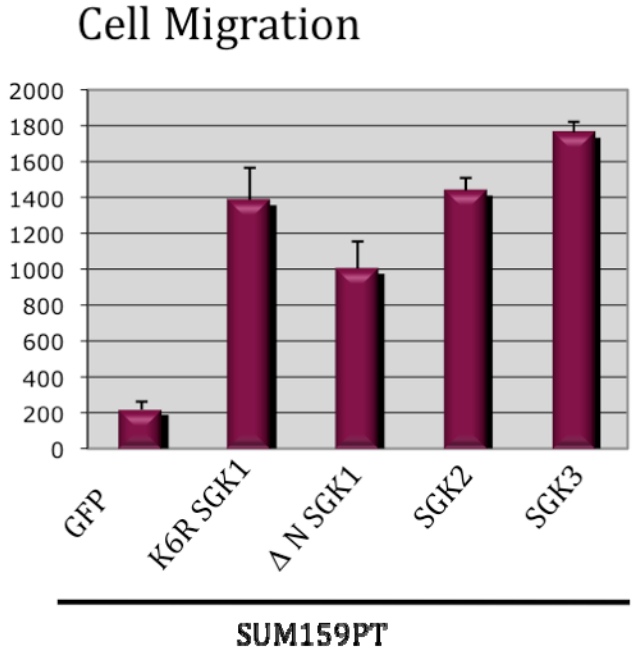
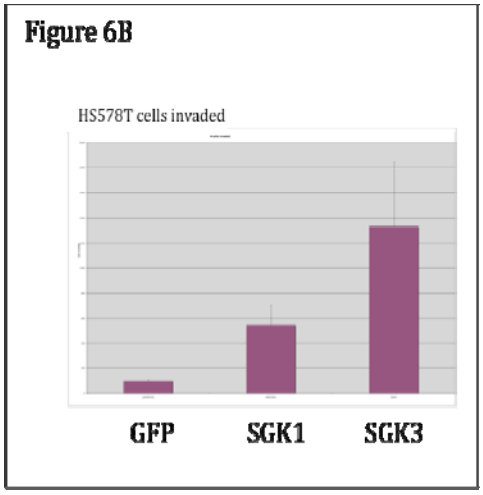
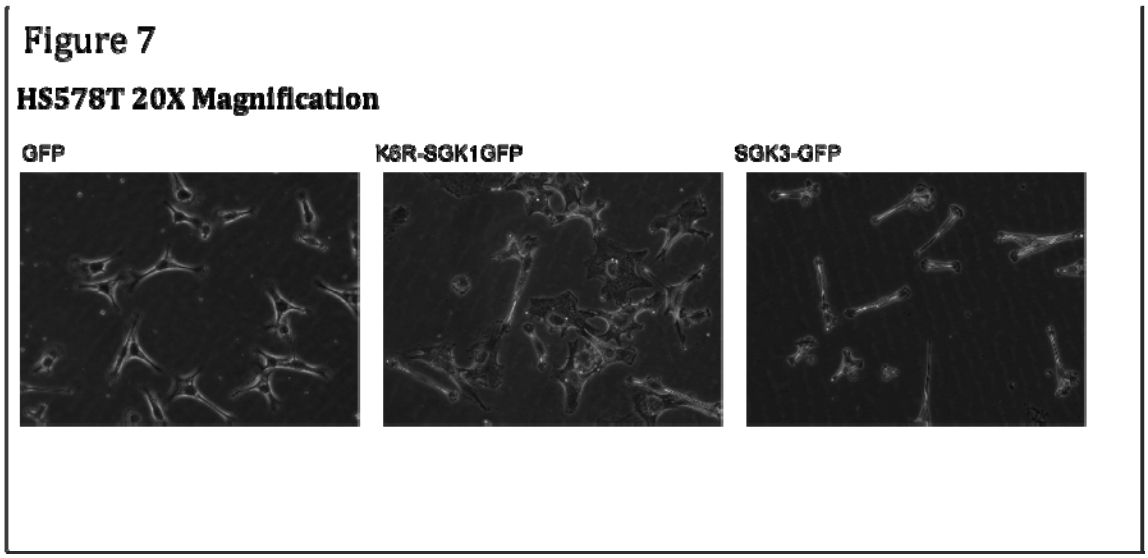


Figure 6A







Current research and future directions

Current experimental efforts have focused upon examining the concordant expression of Akt and SGKs and evaluating whether there is a dominance between the kinases or if there is a switch to choose which kinases drive the PIK3CA pathway, which is the commonly mutated in breast cancer. Efforts to examine breast cancer cells silenced for the SGK isoforms and their motility changes will be our next step to determine the metastatic significance of SGKs.

Key Research Accomplishments

- **Reproducible migratory enhancement by SGK3 in breast cancer cells was discovered**
- **Concordant enhancement of invasion by SGK3 in Matrigel Transwell invasion assays was established**
- **Effective lentiviral knockdown system created with 2 efficient shRNAs towards each SGK1 and SGK3 with corresponding refractory SGK1 and SGK3 overexpression constructs in order to do future rescue experiments to confirm SGK significance in invasive migration of breast cancer.**
- **Efficient PIK3CA overexpression and lentiviral knockdown constructs were made and validated.**
- **Overexpression of PIK3CA breast cancer hot spot mutations, H1047R and E545K, were shown to significantly enhance SGK3 S486 phosphorylation.**

Reportable Outcomes

N/A

Conclusion

SGK1 and SGK3 have been shown in a number of breast cancer cell lines to enhance both migration and invasion using Transwell migrationa and Transwell Matrigel invasion assays. SGK1 and SGK3 shRNAs were subsequently made and validated and will be used to determine the importance of normally metastatic breast cancer cell utilization of SGKs. Additionally PIK3CA overexpression and knockdown constructs were made and validated. The breast cancer hot spot mutations in PIK3CA commonly mutated in breast cancer tumors were shown upon overexpression to activate SGK3 while wild type overexpression caused no change in SGK3 activity. The placement SGK1 and SGK3 downstream of common breast cancer mutations with now demonstrated metastatic characteristics make SGK1 and SGK3 promising future molecular targets for breast cancer therapeutics.

Appendices

N/A

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