The Role of SGK1-NDRG1 Axis in Inflammatory Breast Cancer Stem Cells

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Background

- Inflammatory breast cancer is rare, affecting 2-4% of all women with breast cancer, but makes up a disproportionately high number of breast cancer-related deaths at 10%[1,2,3].
- Previous work from our lab has shown that N-myc downstream regulated gene 1 (NDRG1) regulates cancer stem cells, tumor progression, and brain metastasis[4].
- From the same study, cancer cell lines with silenced NDRG1 expression generated a drastically reduced population of cancer stem cells than control (Fig. 1A and B)[4].
- Research to this point on effective methods of reduction of NDRG1 is lacking. However, examining the pathway of NDRG1, the direct upstream activator serum and glucocorticoid-regulated kinase 1 (SGK1) may represent a potential target for its inhibition (Fig. 1C).
- Our hypothesis is that inhibition of SGK1 will result in the regression of IBC tumors by reducing cancer stem cells and the expression of NDRG1 and phospho-NDRG1.

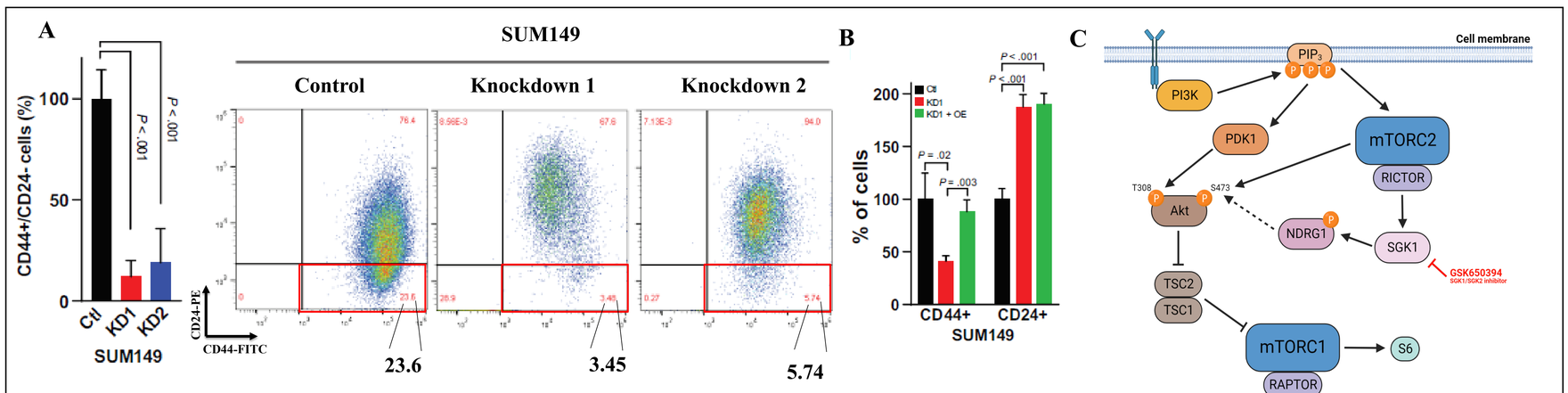

Fig. 1: NDRG1 regulates cancer stem cells. (A) SUM149 cells that had NDRG1 expression knocked down reduced populations of cancer stem cells ($CD44^+CD24^-$) analyzed by flow cytometry [4]. **(B)** Rescue of NDRG1 expression in the knockdown cells partially increased the cancer stem cells [4]. **(C)** The PI3K/AKT pathway highlighting the direct upstream activator of NDRG1, SGK1, and the target inhibitor. KD: knockdown

Methods

- **IC50 Generation**
  - SUM149 cells were treated with varying doses (ranging from 25 x 10^{-5} nM to 25 μM) of GSK650394, an SGK1 inhibitor, over 72 hours.
  - Cell viability was measured by cell titer blue assay.
- **Immunoblot Analysis**
  - Referencing the IC50, a range of concentrations from 125 nM to 30 μM were chosen to treat cells for 1 hour.
  - Expression of pNDRG1, NDRG1, and SGK1 were quantified using immunoblot analysis.
- **Flow Cytometry**
  - Cells were tagged with PE mouse anti-human CD24 and FITC mouse anti-human CD44 and passed through a flow cytometer to measure the number of CD44 high/CD24 low population in control and GSK650394 treated groups.

Results

IC50 of SGK1 Inhibitor GSK650394 in SUM149 cells is 1.02 μM

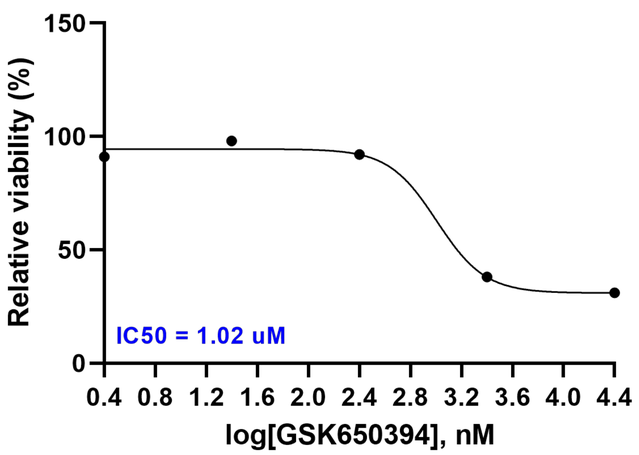

Fig. 2: Following 72 hours of treatment, the IC50 of GSK650394, an SGK1/SGK2 inhibitor, was determined to be 1.02 μM for SUM149 cells.

SGK1 inhibition inhibits expression of NDRG1 and pNDRG1

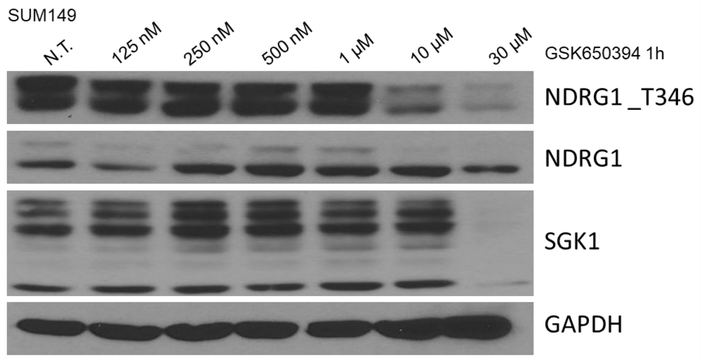

Fig. 3: GSK650394 treatment of SUM149 cells for 1 hour reduced phosphorylation of NDRG1 and SGK1 expression in higher concentrations, specifically 10 and 30 μM doses. N.T. = DMSO vehicle only.

GSK650394 treatments of 10 μM and 30 μM do not reduce cancer stem cell populations

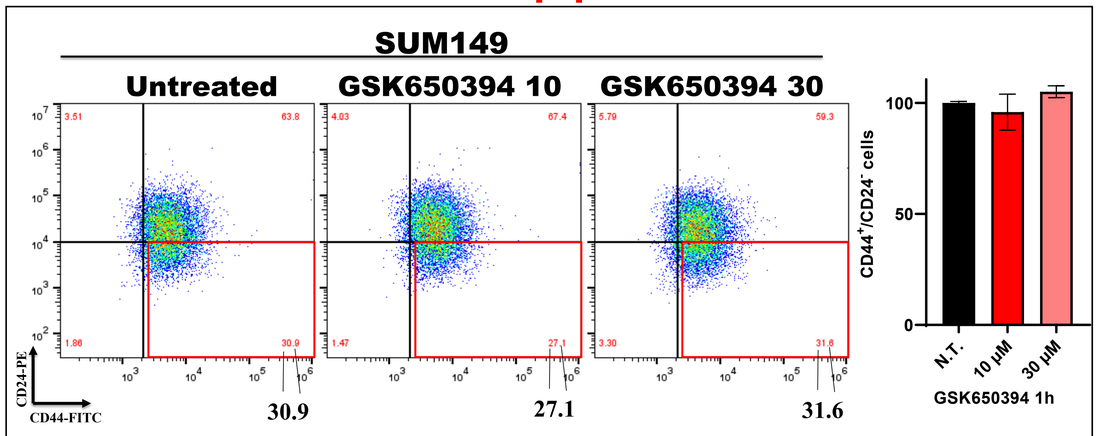

Fig. 4: SUM149 cells treated with 10 μM of GSK650394 for 1 h did not significantly reduce cancer stem cell populations ($CD44^+CD24^-$).

Conclusions

- SGK1 inhibition resulted in reduction of NDRG1/pNDRG1 expression but did not significantly impact cancer stem cell populations.
- Further work is necessary to identify the optimal concentration and time interval of treatment for reducing cancer stem cell populations.
- Beyond identifying optimal dose and duration of drug treatment with GSK650394, this study could be moved in vivo into mouse models to examine how it functions in a biological context.

Acknowledgements

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References

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