Evaluating NHE1 as a Potential Therapeutic Target in Ovarian Cancer

Introduction

This research is focused on investigating the role of the sodium-hydrogen exchanger isoform 1 (NHE1) in the development and progression of ovarian cancer. NHE1 was recently demonstrated to play a role in the regulation of cell proliferation in ovarian cancer (1). The research presented here supports the role of NHE1 in cell proliferation and for the first time implicated NHE1 in the regulation of cell migration in ovarian cancer cell lines. This makes NHE1 a potential therapeutic target in Ovarian Cancer.

Ovarian Cancer accounts for more deaths than any other cancer of the female reproductive system and ranks fifth in cancer deaths among women. A woman's risk of getting ovarian cancer during her lifetime is about 1 in 75 and her chance of dying from ovarian cancer is about 1 in 100. There were an estimated 22,280 new cases of ovarian cancer in 2016 and 14,240 estimated deaths (2.)

Cancer is a family of complex diseases. To better understand the commonalities between different types of cancer, scientists have identified six biological capabilities that cells acquire during cancer development referred to as the hallmarks of cancer (3). All cancers share six common traits that change healthy cells into cancerous cells: 1) Sustaining proliferative signaling, 2) Evading growth suppressors, 3) Activating invasion and metastasis, 4) Enabling replicative immortality, 5) Inducing angiogenesis, and 6) Resisting cell death (3). This work focuses on NHE1 being a contributing factor to two of the hallmarks of cancer, sustaining proliferative signaling and activating invasion and metastasis. See Appendix A for a representation of the Hallmarks of Cancer.

NHE1 is a 12-pass transmembrane protein which functions to exchange one extracellular Na^+ for one intracellular H^+. This transport activity regulates intracellular pH (pH_i), alters extracellular pH (pH_e), and contributes to cell migration. NHE1 has regulatory and transport domains. Amino acids 1-500 comprise the membrane domain and provide transport function. Amino acids 501-815 reside in the cytoplasm and function as the regulatory domain. NHE1 can be activated by increased acid inside the cells or by

phosphorylation of the cytoplasmic regulatory domain. See Appendix B for proposed structure of NHE1 (4).

Protein kinases are enzymes that catalyze the addition of a phosphate group to the proteins. NHE1 is involved in the regulation of all growth and was recently shown to be involved in ovarian cancer (1). This work is focused on four protein kinases that phosphorylate NHE1 at five specific locations: ROCK (RhoA-Associated Kinase) at Threonine 653, RSK (Ribosomal S6 Kinase) at Serine S703, AKT (Protein Kinase B) at Serine S648, and ERK (Extracellular-Signal Regulated Kinase) at Serine S770/S771. Kinase inhibitors block kinase action. If the kinase activates NHE1, the inhibitor should stop that activation. One method to evaluate the roe of kinases in cell signaling is to use pharmaceutical agents called kinase inhibitors. Kinase inhibitors block kinase action. The kinase inhibitors we use are: Cariporide, MK220 (AKT), SCH772984 (ERK), Y2732 (ROCK), and BID1870 (RSK) (5).

The cells that are used in this research are three ovarian cancer cell lines; CAOV-3, SKOV-3, and OVCAR-3. CAOV-3 is an adenocarcinoma cell line from the ovary. It comes from a 54 year old Caucasian woman (6). SKOV-3 is an adenocarcinoma cell line from the ovary. It comes from a 64 year old Caucasian woman (7). OVCAR-3 is an adenocarcinoma cell line from the ovary. It comes from a 60 year old Caucasian woman. (8).

Research Problem

This research hopes to expand our understanding of the role of NHE1 in ovarian cancer by confirming the role of NHE1 in cell proliferation and in the regulation of cell migration.

Hypothesis

The inhibition of NHE1 transport activity by cariporide or the inhibition of NHE1 activation by specific protein kinase inhibitors will decrease cell proliferation and cell migration in ovarian cancer cell lines.

Methods

Cell Culture: Cells are grown in lab and cultured each day. Different cell types are grown in different media. Media acts as nutrients to the cells. The CAOV-3 cell line is cultured in DMEM media. The OVCAR-3 cell line is cultured in RMPI media. The SKOV-3 cell line is cultured in McCoys media. All cells were supplements with 10% fetal bovine serum.

Proliferation Assay: An XTT Proliferation Assay is a spectrophotometric assay in which live cells conduct a reduction reaction that produces an increase in pigment that can be measured as a change in absorbance. In this assay we seed 5,000 cells into a 96-well plate consisting of eight rows and 12 columns. The first three columns are media blanks, which are used as a negative control for pigment produced in the absence of cells. The next nine columns are cells plus media. The cells are grown in 0.5% serum, 10% serum, 10% serum plus cariporide, and 10% serum plus specific kinase inhibitors. The plate is incubated for 72 hours and then a working reagent is added and the plate is put back into the incubator for two hours. While the plate is in the incubator the reduction reaction occurs. The corresponding color change only happens when there are cells present and is proportional to the number of cells. After two hours the absorbance is read using a plate reader. The data is then analyzed and graphed.

Migration Assay: An Electric Cell-substrate Impedance Sensing (ECIS) Migration Assay takes advantage of the lipid cell membrane's ability to act as a resistor to current flow. The ECIS arrays contain gold electrodes that allow the measurement of resistance based upon the number of cells attached to the electrode. To initiate the experiments, 400,000 cells are seeded in each well of the electrode. The cells are allowed to grow to high 100% confluence. Once cells reach confluence, the media on the cells is changed to the appropriate experimental media and cells are incubated for six hours. A high current pulse is then sent through the electrode to kill all of the cells attached to the electrode surface. Changes in resistance are then measured over the following 8 to 10 hours. This assay evaluates the rate of cell migration back onto the electrode. The data is then analyzed and graphed.

Results

Proliferation Assay: Proliferation was measured using an XTT proliferation assay as describe in the methods. A proliferation assay was performed for CAOV-3 cells (Figure 1), SKOV-3 cells (Figure 2), and OVCAR-3 cells (Figure 3). A change in growth conditions from 0.5% serum to 10% serum increased cell proliferation 2 – 3 fold in each cell type. This increase in proliferation was blocked by the NHE1 inhibitor cariporide (10μM). For each cell type the protein kinase inhibitors MK220 (AKT), SCH772984 (ERK), Y2732 (ROCK), and BID1870 (RSK) were used to evaluate the role of growth signaling to NHE1 in the stimulation of cell proliferation. In both CAOV-3 and SKOV-3 cells all four kinase inhibitors decreased the rate of cell proliferation with the RSK inhibitor BID1870 had the greatest impact. In OVCAR-3 cells inhibition of AKT by MK220 induced the greatest reduction of cell growth and inhibition of ROCK by Y27632 had no impact on proliferation.

Migration Assay: To evaluate the role of NHE1 in cell migration an ECIS cell migration assay was performed on the SKOV-3 cell line (Figure 4). The rate of migration was measured as a change in impedance over time as described in the methods. A substantial increase in cell migration rate occurred in the presence of 10% serum. This increase in migration rate was decreased both by cariporide (10 μM) and the RSK inhibitor BID1870. proliferation.

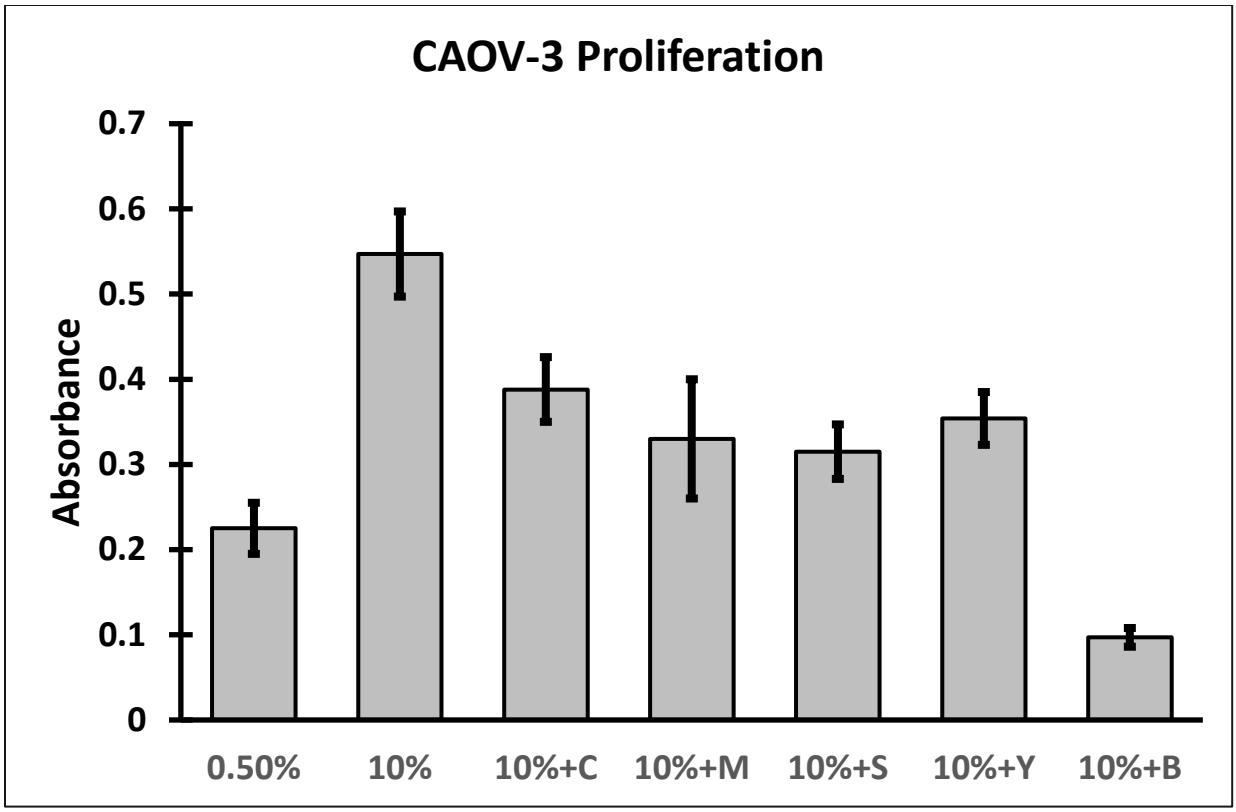

Figure 1. CAOV-3 Proliferation Assay. Cell growth was measured for 72 hours in CAOV-3 cells. The increase in growth in the presence of 10% serum was inhibited by the NHE1 specific inhibitor cariporide(C). Additionally, the protein kinase inhibitors MK220 (M, AKT), SCH772984 (S, ERK), Y2732 (Y, ROCK), and BID1870 (B, RSK) each inhibited cell growth. Inhibition of RSK by BID1870 induced the greatest reduction in cell proliferation.

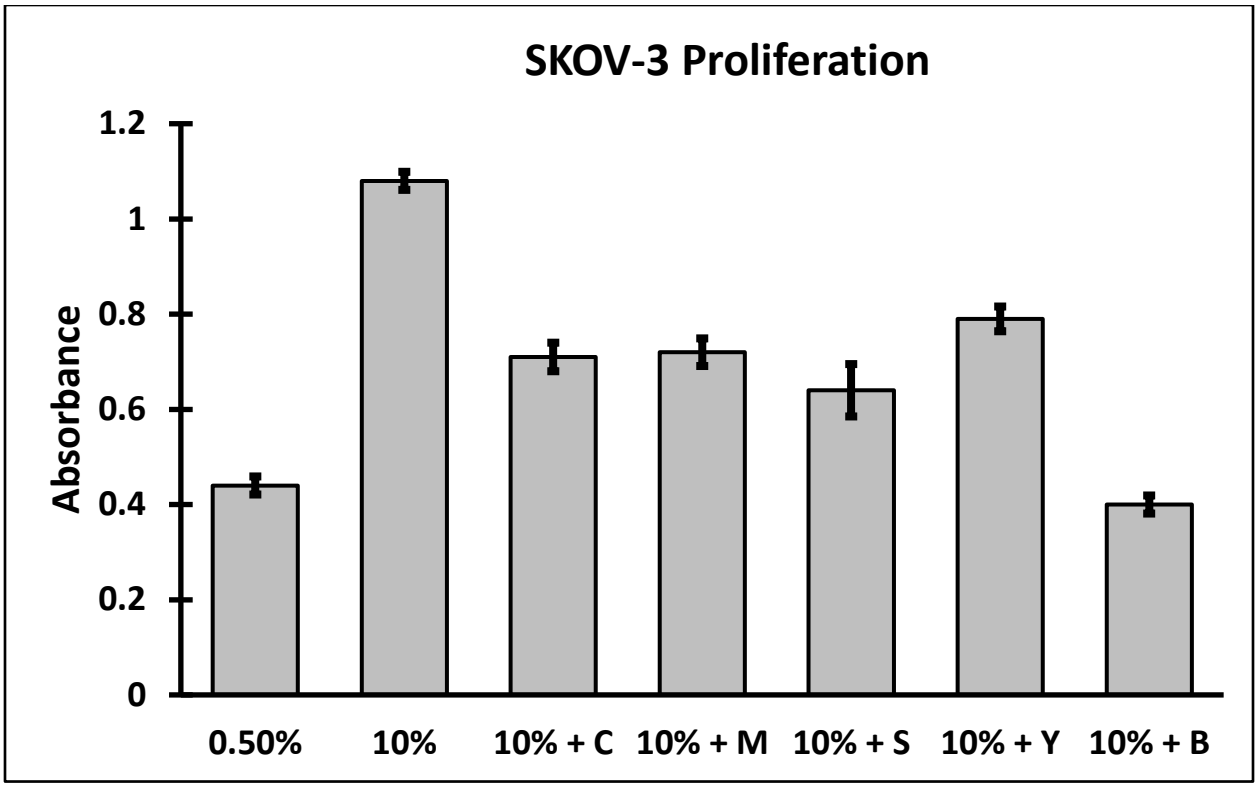

Figure 2. SKOV-3 Proliferation Assay. Cell growth was measured for 72 hours in SKOV-3 cells. The increase in growth in the presence of 10% serum was inhibited by the NHE1 specific inhibitor cariporide(C). Additionally, the protein kinase inhibitors MK220 (M, AKT), SCH772984 (S, ERK), Y2732 (Y, ROCK), and BID1870 (B, RSK) each inhibited cell growth. Inhibition of RSK by BID1870 induced the greatest reduction in cell proliferation.

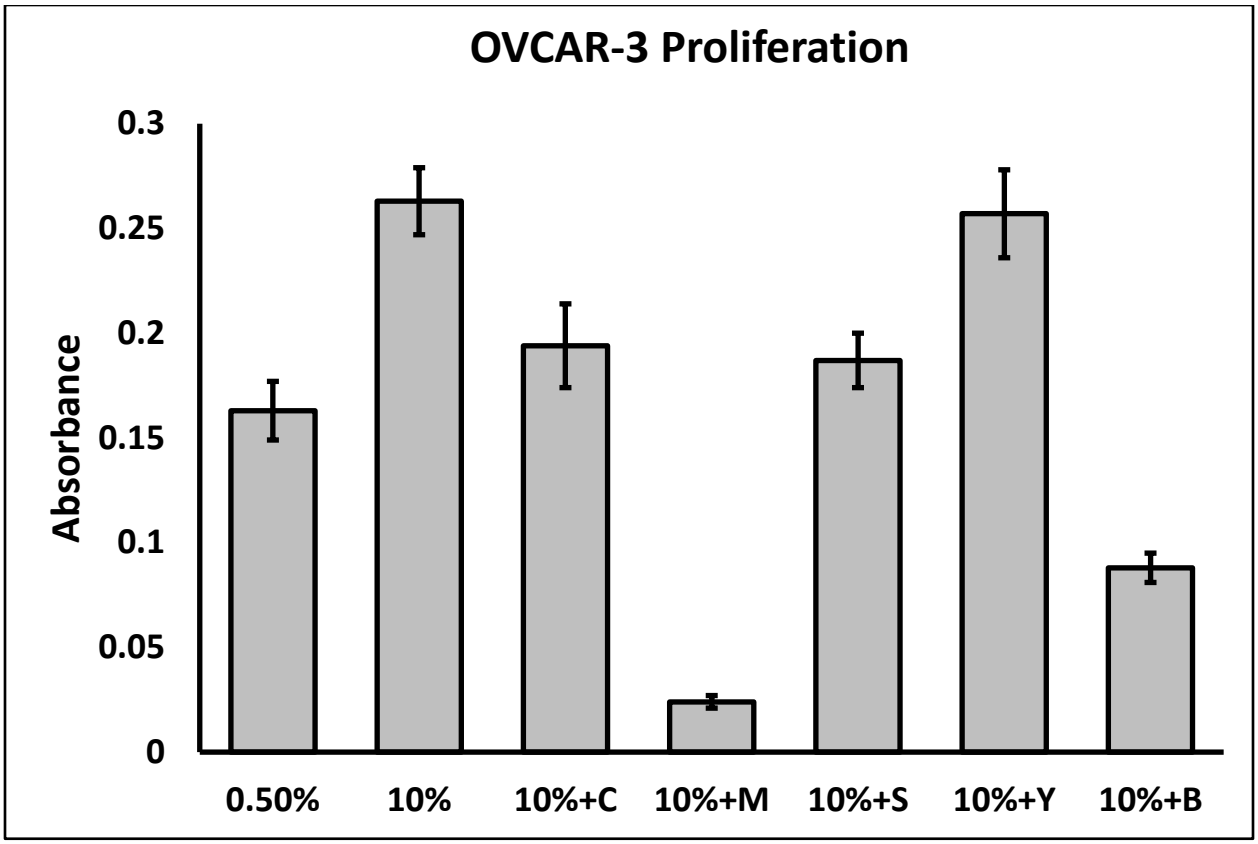

Figure 3. OVCAR-3 Proliferation Assay. Cell growth was measured for 72 hours in OVCAR-3 cells. The increase in growth in the presence of 10% serum was inhibited by the NHE1 specific inhibitor cariporide(C). Additionally, the protein kinase inhibitors MK220 (M, AKT), SCH772984 (S, ERK), Y2732 (Y, ROCK), and BID1870 (B, RSK) each inhibited cell growth. Inhibition of AKT by MK220 induced the greatest reduction in cell proliferation. Inhibition of ROCK by Y27632 had no impact on proliferation.

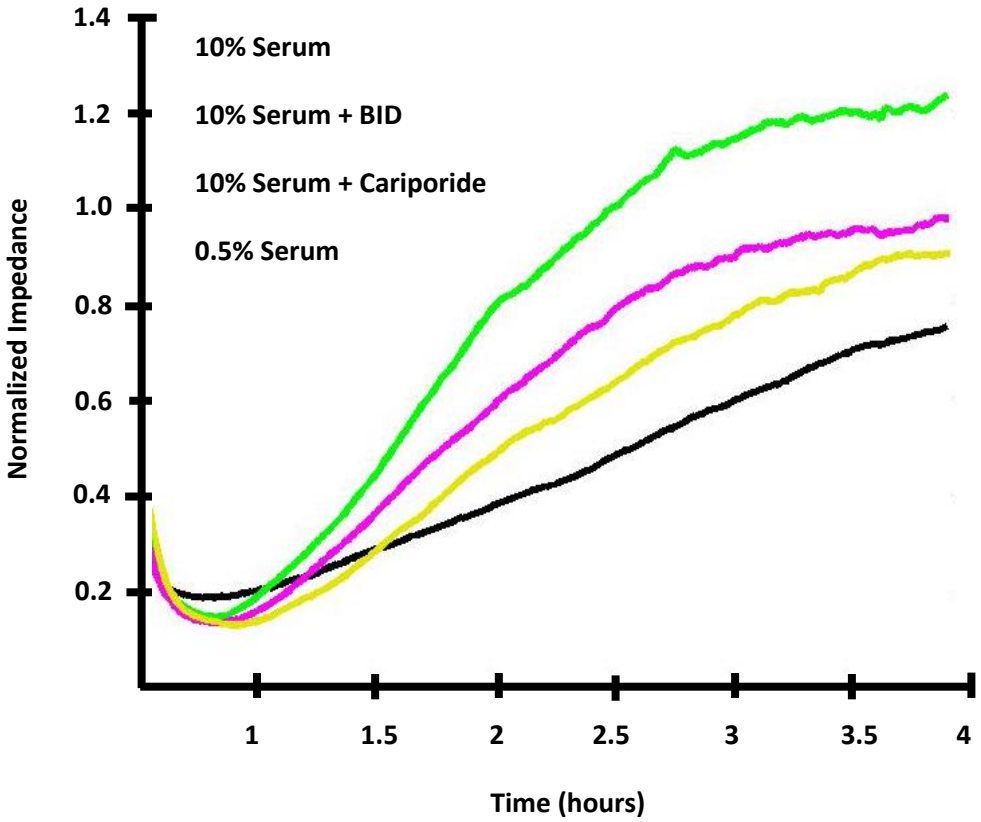

Figure 4. SKOV-3 Migration Assay. Cell growth was measured for 72 hours in SKOV-3 cells. The increase in migration in the presence of 10% serum was inhibited by the NHE1 specific inhibitor cariporide(C). Additionally, the protein kinase inhibitor BID1870 (BID, RSK) inhibited cell migration.

Discussion

In each of the three cells types, CAOV-3, SKOV-3, and OVCAR-3 an increase in serum from 0.5% to 10% increased cell growth. The NHE1 inhibitor, cariporide decreased proliferation in all three cells types confirming recently published results that NHE1 activity contributes to cell proliferation in ovarian cancer cells (1). These experiments also evaluated to role of four different kinases known to activate NHE1 in the stimulation of cell proliferation by using specific kinase inhibitors. The kinase inhibitor showing the greatest impact in the SKOV-3 and CAOV-3 cells was BID1870, an inhibitor of RSK. In OVCAR-3 cells a different inhibition pattern was identified with the Rock inhibitor (Y27632) showing no inhibition and the AKT inhibitor MK2206 having the greatest impact. In the cell migration experiments, inhibition of migration in the SKOV cells, an increase in serum from 0.5% to 10% dramatically increased cell migration rate. This increase in migration was dramatically decreased by the presence of the NHE1 inhibitor cariporide indicating a role for NHE1 in the regulation of cell migration in ovarian cancer cells for the first time. Additionally, the RSK inhibitor BID1870 also dramatically decreased cell migration.

These results confirms the importance of NHE1 in cell proliferation in ovarian cancer and for the first time implicates NHE1 in cell migration in these cells. These data indicate that NHE1 may serve as a potential therapeutic target in Ovarian Cancer treatment.

References

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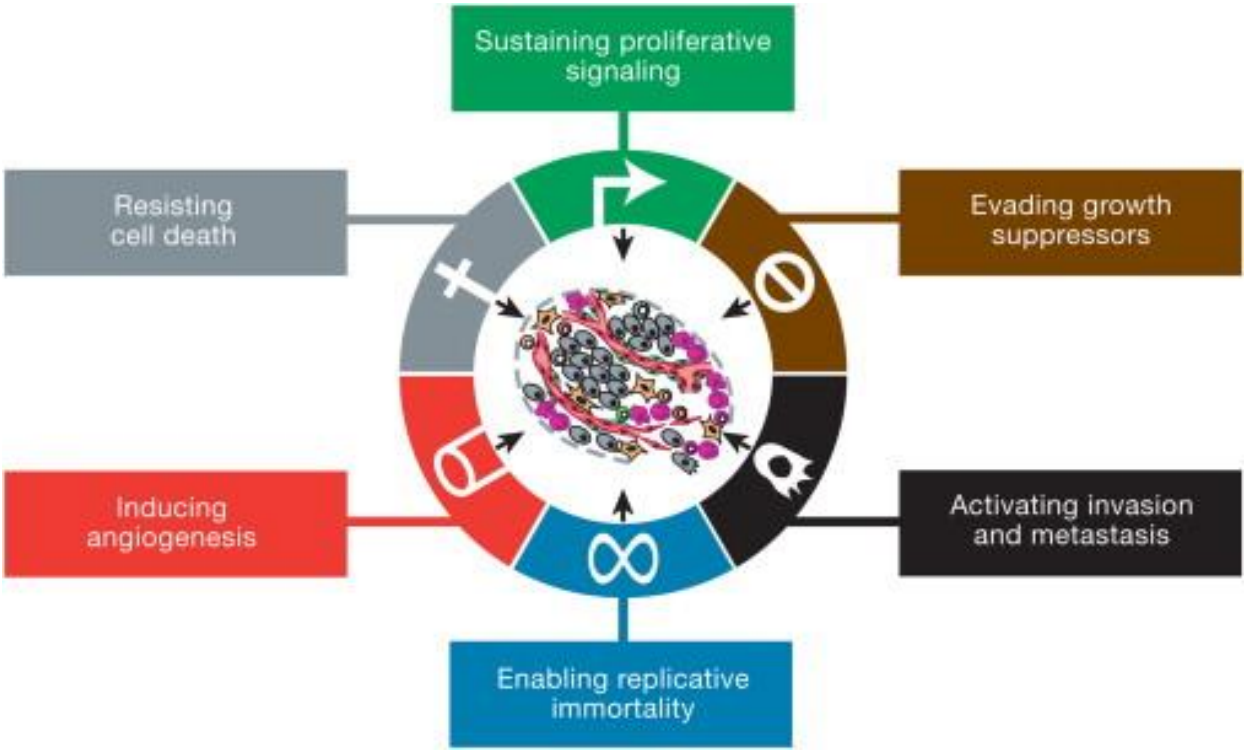
Appendix A

The Hallmarks of Cancer as described by Hanahan and Weinberg. (3)



Appendix B

The proposed Structure of NHE1 with key phosphorylation sites indicated on cytoplasmic regulatory domain.