Lankenau Institute for Medical Research

Annual Progress Report: 2010 Formula Grant

Reporting Period


Formula Grant Overview

The Lankenau Institute for Medical Research received $175,518 in formula funds for the grant award period January 1, 2011 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1:  Project Title and Purpose

Disulfides to Modulate Thiol Homeostasis in Human Colon Cancer Cells – The efficacy of most chemotherapeutic agents and radiation in cancer cells may be limited due to its detoxification by intracellular glutathione (GSH). Our preliminary results have demonstrated that hydroxyethyl disulfide (HEDS) depletes GSH and increases the response of glucose deprived cancer cells to radiation. However, HEDS treatment has only a limited success in increasing the response of rat tumor xenograft to chemotherapy since it is metabolized faster by cells with glucose. The next step in our drug discovery effort is to screen disulfides with different chemical structures that will identify disulfides with better stability than HEDS. These studies will also determine the impact of a low glucose microenvironment, which induces resistance to therapy, on the disulfides mediated depletion of GSH in human colon cancer cells.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

Recent studies from our group have demonstrated that oxidative pentose cycle deficient or glucose deprived rodent cells and human cancer cells are susceptible to hydroxyethyl disulfide (HEDS) mediated decrease in protein and non protein thiols and sensitization to radiation. Based on these in vitro studies, HEDS was expected to be advantageous in sensitizing solid tumors to radiation since it will specifically sensitize glucose deprived hypoxic tumor cells that are resistant to cancer therapy. However, our preliminary studies (results not shown) have demonstrated that this strategy has only a limited success in increasing the response of rat tumor xenograft to radiation and chemotherapy in vivo since it is metabolized faster by cells with glucose. The next step in our drug discovery effort is to screen drugs with a similar functional (disulfide) group but with different chemical structures to determine the structure activity relationship. This approach will not only identify disulfides with better stability than HEDS but
also will determine the impact of a low glucose microenvironment, which induces resistance to therapy, on the disulfide mediated depletion of glutathione (GSH) in human colon cancer cells.

The planned pilot study will screen twenty disulfides with different chemical structures for their efficacy to alter the redox status of glutathione, and to determine the detoxification of these compounds by human colon cancer cells in the presence and absence of glucose in vitro. The major focus of this project is to determine whether one or more of these disulfide compounds have a better thiol modulation property than hydroxyethyl disulfide.

**Specific Aim 1:** Determine the extent of conversion of disulfides with different chemical structures into sulfhydryl compounds (i.e., detoxification) by human colon cancer cells in the presence and absence of glucose.

The conversion of disulfides into sulphydryl will be measured by quantifying free thiols in the extracellular medium produced by bioreduction of disulfides using HPLC/electrochemical detection and/or 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) assays.

**Specific Aim 2:** Determine the effect of these disulfides on intracellular thiol redox in the presence and absence of glucose.

To quantify the effect of these disulfides on intracellular thiols, the cellular extract prepared by sulfosalicylic acid lysis buffer will be used for the analysis of thiols using HPLC/electrochemical detection and/or 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) assays.

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**Expected Research Outcomes and Benefits**

Low oxygen (hypoxia) is prevalent in most solid tumors and plays a major role in the poor outcome of cancer therapy. Hypoxic (low oxygen) cells in solid tumors are also glucose deprived due to disorganized vasculature. Glucose deprived cells are resistant to radiation and certain chemotherapeutic agents. It raises the importance of identifying agents that can target glucose deprived hypoxic cancer cells in solid tumors. Glutathione, an antioxidant, plays a major role in the survival of cancer cells in an oxidative stress environment, which is likely to be caused by low glucose and low oxygen in solid tumors. In contrast, certain disulfides deplete glutathione causing loss of function of proteins and cell death under certain conditions. The FDA has
recently approved a disulfide called glutathione disulfide mimetic (NOV-002) for phase 2/3 clinical trial for cancer in the US. This compound has shown some limited but significant improvement when combined with chemotherapy. NOV-002 is not membrane permeable, which may be the reason for its limited success. We have demonstrated that another disulfide hydroxyethyl disulfide (HEDS) is membrane permeable and specifically targets glucose deprived cancer cells in vitro. These studies suggested that the effectiveness of disulfides in cancer therapy is dependent not only on the disulfide functional group but also on the chemical structure of the compound. Our project will determine the structure activity relationship of several disulfide compounds in targeting glutathione, which plays a major role in the survival of cancer cells during oxidative stress induced by the tumor microenvironment, chemotherapeutic agents and radiation. The planned studies will 1) screen twenty disulfides with various chemical structures in comparison to hydroxyethyl disulfide and NOV-002 for their effectiveness in depleting glutathione and 2) determine the detoxification of these compounds by human colon cancer cells in the presence and absence of glucose. This approach will not only identify disulfides better than HEDS, but will also determine the human colon cancer cells’ ability in the detoxification of these disulfides under glucose deprivation found in solid tumors.

**Summary of Research Completed**

During the first six months of the funding period, we tested fourteen of the following disulfide compounds (1 – 20) for their detoxification (Specific Aim 1) by HCT116 and HT29 human colon cancer cells in the presence and absence of glucose.

1) 3-Nitrophenyl disulphide. 2) Bis (4-Methoxyphenyl) disulphide. 3) Di-tert-butyl disulphide. 4) Allyl sulphide 5) Di butyl disulphide. 6) Di ethyl disulphide. 7) Dipropyl disulphide. 8) Isopropyl disulphide. 9) Methyl propyl disulphide. 10) P-tolyl disulphide. 11) Phenyl disulphide. 12) Phenyl acetyl chloride. 13) Sec-Butyl disulphide. 14) 3,3’Dihydroxydiphenyl disulphide.15) Di methyl disulphide. 16) 2-Nitro-P-tolyl disulphide. 17) 4-Nitrophenyl disulphide. 18) Bis (2-Nitrophenyl) disulphide. 19) Di Allyl Sulphide. 20) Di benzyl disulphide.

**Methods and Designs:** We used two established human colon cancer cells (HCT116, HT29) obtained from ATCC, USA. All experiments were carried out at a single concentration of cells (1 million) grown in a 60mm dish with DMEM medium with 10%FCS, 1% penicillin/streptomycin (P/S) and 20 mM HEPES. These cells were rinsed four times with DMEM without glucose (hereafter called as DMEM-G) to remove the residual glucose in the dish. DMEM-G rinsed cells were incubated for 4 hours with one ml of DMEM-G containing 0 and 25mM glucose. After 4hr glucose starvation, cells were exposed to different concentrations of disulfide for 1, 2, 3, 8, and 24hrs at 37°C in a 5% CO₂ incubator. The conversion of disulfides into sulphydryl by the human colon cancer cells was measured by quantifying the sulphydryl compound in the extracellular medium produced by bioreduction of 14 disulfides using HPLC/electrochemical detector or dithiobis nitrobenzoic acid reagent (DTNB). To quantify the disulfide metabolism, 0.5 ml of extracellular medium was mixed with 0.5 ml of 100mM sulfosalicyclic acid (SSA) lysis buffer in microfuge tubes and centrifuged in a microfuge. For HPLC/EC assay, the medium extract was analyzed using HPLC system consisting of a single pump, autosampler, guard cell, 5010 analytical cell and Colouchem III (ESA, USA). Diluted sample (10µl) was loaded onto a C18 column and run in an isocratic mode using a mobile phase.
with 50mM phosphate, pH 2.7, 0.05mM octane sulfonic acid and 2.2% acetonitrile. For 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) assay, 150µl of the extract was mixed with 1200µl of phosphate buffer and 150µl of 10mM DTNB. The optical density of this reaction mixture was measured at 412nm and the concentration calculated using an extinction coefficient of 1.36x10⁴ for reduced DTNB.

Results: In Specific Aim 1, we have proposed to determine the extent of conversion of several disulfides into monothiols (i.e., detoxification) by human colon cancer cells HCT116 and HT29 in the presence and absence of glucose. Towards this goal, we first determined the metabolic conversion of a single concentration (5mM) of the first fourteen disulfides (see the list above) into monothiols in the presence and absence of glucose. The results showed that compound 10 (tolyl disulfide) and compound 11 (phenyl disulfide) were converted into 140 and 540µM monothiols, respectively, by HCT116 cells after 2 hours incubation in the presence of glucose (Figure 1). The results have also shown that compounds 10 and 11 were converted into 120 and 500µM monothiols, respectively, by HT29 cells after 2 hours incubation in the presence of glucose (Figure 2). However, these two compounds were not effectively converted into monothiols in the absence of glucose (Figures 3 and 4). However, all the other compounds neither showed effective metabolic conversion nor glucose dependency similar to that observed for compounds 10 and 11 (Figures 1, 2, 3, 4).

We have also measured the metabolic conversion of these fourteen compounds after up to 24 hours incubation in the presence of glucose to determine whether some of these compounds can be converted into monothiols after longer incubation with human cells. The results have shown incubation time dependent metabolic conversion of some of these disulfides by HCT116 cells in vitro (Figures 5, 6, 7, 8, 9). In particular, these fourteen drugs have been converted into monothiols after 24 hours incubation (Figure 9). However, the bioreduction of these compounds into monothiols was significantly altered after 1, 2, 3 and 8 hours incubation with HCT116 cells in the presence of glucose (Figures 5, 6, 7, 8, 9). Consistent with the results in Figures 1 and 3, tolyl disulfide (compound 10) and phenyl disulfide (compound 11) were the only two disulfides converted into monothiols by HCT116 effectively at lower incubation times in the presence of glucose.

We previously demonstrated that hydroxyethyl disulfide was metabolized by human and rodent cells very rapidly in the presence of glucose. We therefore determined the stability of these two new compounds (tolyl disulfide and phenyl disulfide) by comparing the metabolic conversion of different concentrations (0, 0.5, 1, 2, 3, 4mM) of these compounds into monothiols by HCT116 cells with that of hydroxyethyl disulfide. The results have demonstrated that 4mM tolyl disulfide is converted into 114µM monothiol by HCT116 cells after 2 hour incubation in the presence of glucose (Figure 10). On the other hand, 4mM phenyl disulfide is converted into 516µM monothiols by HCT116 cells after 2 hour incubation in the presence of glucose (Figure 11). The metabolic conversion of these two compounds is lower than the metabolic conversion of hydroxyethyl disulfide (2237µM) under similar conditions (Figure 12). However, all these three compounds require glucose for metabolic conversion into monothiols (Figures 10, 11, 12). The higher stability of these two new compounds compared to hydroxyethyl disulfide suggests that these compounds may be more effective as a chemotherapy agent both in vivo and in vitro.
Significance
The results have demonstrated that at least two of the fourteen disulfides screened so far have better stability than HEDS and are likely to have better efficacy in killing glucose deprived cancer cells in solid tumors.
Figure 1: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 2 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 2, 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 2: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 2 hour incubation with human colon cancer cells HCT116 in the absence of glucose. The results have demonstrated that compounds 2, 10 and 11 are also not metabolized in the absence of glucose in vitro.

Figure 3: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 2 hour incubation with human colon cancer cells HT29 in the presence of glucose. The results have demonstrated that compounds 2, 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 4: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 2 hour incubation with human colon cancer cells HT29 in the absence of glucose. The results have demonstrated that compounds 2, 10 and 11 are also not metabolized in the absence of glucose in vitro.
Figure 5: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 1 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 2, 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 6: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 2 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 7: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 3 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 8: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 8 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 1,2,6,7,8,9,10 and 11 are metabolized better than the other compounds tested in vitro.
Figure 9: Quantification of detoxification/bioreduction of different disulfide compounds (5mM) after 8 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that compounds 1, 2, 6, 7, 8, 9, 10 and 11 are metabolized better than the other compounds tested in vitro.

Figure 10: Quantification of detoxification/bioreduction of different concentrations of tolyl disulfide (0, 0.5, 1, 2, 3, 4mM) after 2 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that tolyl disulfide showed a glucose dependent conversion into monothiols at all concentrations tested in vitro.

Figure 11: Quantification of detoxification/bioreduction of different concentrations of phenyl disulfide (0, 0.5, 1, 2, 3, 4mM) after 2 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that phenyl disulfide showed a glucose dependent conversion into monothiols at all concentrations tested in vitro.

Figure 12: Quantification of detoxification/bioreduction of different concentrations of hydroxyethyl disulfide (0, 0.5, 1, 2, 3, 4mM) after 2 hour incubation with human colon cancer cells HCT116 in the presence of glucose. The results have demonstrated that hydroxyethyl disulfide showed a glucose dependent conversion into monothiols at all concentrations tested in vitro.
Research Project 2: Project Title and Purpose

Role of TIMP-4 in Breast Cancer Assessment and Treatment - The planned work will assess the use of a new therapeutic agent to target the triple-negative breast cancers (TNBC) identified as highly aggressive even when diagnosed at a small size. We know from previous work that elevated levels of tissue inhibitor of metalloproteinases-4 (TIMP-4) in TNBC are associated with poor prognosis for disease-free survival. These tumors are highly aggressive and difficult to treat due to lack of targeted therapy and/or resistance to standard therapy. A new agent, which blocks the down-stream effects of TIMP-4, can be the first agent to improve response rates and thereby survival among TNBC patients, a group that contributes disproportionately to the breast cancer associated death rate.

Anticipated Duration of Project

1/1/2011 – 12/31/2011

Project Overview

The objective for this project is to obtain new information regarding the effects of elevated TIMP-4 levels on tumor behavior. The new information could result in changes in clinical practice to provide a better treatment plan for patients with triple-negative but TIMP-4 positive breast cancer, a group that currently has few effective treatment options available.

In this project we will assess circulating TIMP-4 levels in breast cancer patients by analyzing blood samples collected approximately 3-4 weeks after surgical removal of the tumor (Specific Aim 1). Plasma from the collected blood samples will be analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Circulating levels of TIMP-4 will be assessed at several times throughout the initial treatment regimen.

We will also employ a standard animal model of breast cancer to obtain preclinical “proof-of-concept” that blocking TIMP-4 induced signaling can prevent breast tumor growth and progression (Specific Aim 2). The animals will have slow-release pellets containing human TIMP-4 protein implanted into the mammary fat pad (mfp) followed by inoculation of human breast cancer cells. Once palpable tumors have formed, animals will be treated with PI3K-inhibitors alone or in combination with a chemotherapeutic agent. Changes in tumor growth and progression will be followed and compared to vehicle alone. Obtained results will determine if the new PI3K inhibitors could be tested in clinical trials for treatment of TNBCs with elevated TIMP-4.

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Expected Research Outcomes and Benefits

We predict that we will obtain data confirming our initial observation that circulating levels of TIMP-4 are independent of tumor burden and therefore will remain at a similar level post-surgery as pre-surgery (Specific Aim 1). This will attest to the need for TIMP-4 targeted therapy. The results obtained from Specific Aim 2 will provide “proof-of-concept” for our hypothesis that downstream signaling of TIMP-4 can be an effective approach to treat patients with TNBC and elevated TIMP-4 levels. The obtained results will be the foundation of a new R21 application. Further testing to identify an agent that can block the downstream signaling in patients with elevated TIMP-4 levels could help reduce the aggressive behavior and render the tumors more susceptible to conventional chemo- and radiation therapy.

Summary of Research Completed

During the past six months the current project, which consist of two specific aims, has been initiated and are currently on going.

In Specific Aim 1 we proposed to study the circulating levels of TIMP-4 in breast cancer patients prior to surgery and as they, if deemed appropriate by their medical oncologist, are treated with chemotherapy. This aim is based on our previous published observation that breast cancer patients with elevated levels of TIMP-4 are at higher risk of recurrence, indicating a more aggressive tumor type that might also be less sensitive to current standard treatments. The recurrence risk is higher among the so-called “triple-negative” breast cancers (TNBCs), a type of breast cancer where chemotherapy is the only available treatment option. Our previous research had also demonstrated that some healthy women, independent of age, have TIMP-4 levels as high as those found in women with recurrent and/or metastatic breast cancer. Based on these observations we generated a protocol, approved by our local Institutional Review Board (IRB), to assess the effects of tumor burden on circulating TIMP-4 levels. The approved protocol allows us to enroll patients seeking care at Lankenau Medical Center (LMC) for their breast cancer diagnosis.

During the first six months of the project, all patients diagnosed with breast cancer and recommended adjuvant chemotherapy have been asked to participate in the IRB-approved study. Of all breast cancer patients, only those with larger tumors (i.e., larger than 2cm in the largest dimension) or TNBC patients with tumors larger than 0.5 cm are usually treated with chemotherapy after surgical resection of the primary tumor. Of the ~50 patients that fit the selection criteria for chemotherapy 8 patients choose to enroll in the study. We obtained blood samples, drawn into an EDTA-coated (“purple top”) tube for plasma collection. Samples were drawn prior to 1) initiating therapy, 2) prior to each additional cycle of therapy and 3) at follow-
up visits. Samples were de-identified by assigning a study number before transfer of samples to the laboratory. Samples were kept on ice and handled at 4°C at all times.

After the plasma had been separated, aliquots were transferred into new tubes and immediately frozen and stored at -80°C until analysis. At time of analysis, each tube was allowed to thaw on ice and then gently mixed before added into the commercial ELISA coated with a TIMP-4 specific capture antibody (R&D Systems). Samples were added along with known amounts of recombinant human TIMP-4 to generate a standard curve for assessment of TIMP-4 levels in the patient plasma (Figure 1). Following the instructions from the manufacturer, unbound material was washed using the provided wash buffer followed by addition of a conjugated second TIMP-4 specific antibody and substrate for color development. The absorption at 450nm, obtained using a BIO-TEK Synergy HT plate reader, was used to determine the amount of circulating TIMP-4 per ml of plasma (Figure 1).

Of the consented patients, three had consented to another protocol where we assess TIMP-4 levels in plasma, using the same technique, immediately prior to surgery (Figure 2, pt’s A - C). These three patients received Taxotere and cyclophosphamide (TC) starting 4, 7 and 9 weeks after initial surgery, respectively. In these three patients, the TIMP-4 levels increased between time-of-surgery and the start of chemotherapy. The increase ranged between 1.7- and 2.2-fold the initial value.

These early results suggest that the TIMP-4 levels are not exclusively dependent of the presence of tumor mass. The levels continued to increase during the first cycles of therapy and returned to or slightly below pre-surgery levels at follow-up visits (F/U) or at later cycles (Pt B [Figure 2], received 6 cycles). We are continuously following these patients.

The remaining patients had their surgery at other hospitals but choose LMC for the medical oncology treatments. The plasma levels among these patients were elevated, as compared to healthy controls in the same age range [results from another on-going TIMP-4 study] and demonstrated a similar increase during the first cycle(s) followed as seen in the group we followed from time of surgery. The TIMP-4 levels decreased at later treatment cycles or at first follow-up visit in the same manner we had observed for those we followed since surgery. Though early in the study, it seems that the decline in TIMP-4 levels is slow but obtainable in TC-treated patients. An interesting observation was made for patient F (Figure 2), who had an adverse reaction to TC and was switched after two cycles to Adriamycin/cyclophosphamide (AC) that resulted in a dramatic drop in TIMP-4 levels. We are following all patients through their follow-up visits at LMC and continue to enroll new patients that are eligible as per the approved protocol.

In Specific Aim 2, we proposed to study the effects of blocking the TIMP-4 induced growth and survival signaling by using new PI3K-inhibitors. We have submitted an animal protocol for the proposed animal study to our Institutional Animal Care and Use Committee (IACUC) for review. This protocol is currently under revision and will hopefully be approved next month. As part of this specific aim we have obtained the new PI3K-inhibitor GDC-0941 (in clinical trails by Genentech for solid tumors other than TNBC) and tested it in several human breast cancer cell lines to determine the lowest effective dose.
We have focused on two cell-lines, MDA-MB-435S and MDA-MB-468, both from ATCC. These two human breast cancer cell-lines were established from triple-negative breast cancer patients and are routinely cultured in Leibovitz’s L-15 medium supplemented with 10% fetal bovine serum. We have chosen these cell-lines to compare the effects of GDC-0941 because we had performed the initial work with MDA-MB-435S that we have shown to have high levels of the tetraspanin CD63. This cell surface molecule is the binding partner of TIMP-4 and induces growth stimulation and survival signaling when TIMP-4 is bound to its extracellular domain. However, there is some controversy in the literature regarding the tissue origin of this line and in an attempt to prevent critique of the work based on choice of cells used, we have tested MDA-MB-468 for CD63 expression and found it be almost as high as MDA-MB-435S. Using a line with high CD63 expression will facilitate the detection of changes in behavior and signaling after TIMP-4 exposure.

In an initial test we used GDC-0941 alone to treat cells at 100, 50 or 20 µM. These results (data not shown) demonstrated that GDC-0941 at the two higher doses causes massive cell death within 24 hours. The lowest dose tested (20µM) also caused some cell death over time, but cells remained viable but not proliferating during the time course. To have some dose suggestions for the IACUC protocol submission, we then lowered the GDC-0941 dose further and tested it in combination with current standard chemotherapy agents or as single agent in cell cultures under normal or elevated TIMP-4 conditions.

In brief, we treated cells, seeded at equal density, with 2nM TIMP-4, which corresponds to a level of circulating TIMP-4 associated with early recurrence and progression to metastatic disease among breast cancer patients. In parallel cultures we added either 1) 10µM GDC-0941, a previously published concentration for cell culture use, or 2) TC at 10nM Taxotere and 0.5mM cyclophosphamide, or 3) TC and GDC-0941 in combination.

Cell growth was followed over a two-week period and results graphed (Figure 3). The growth stimulatory effect of TIMP-4 was stronger in MDA-MB-468 cells than in MDA-MB-435S but the response to GDC-0941 and TC similar in both lines. We are currently testing even lower concentrations to address concerns from the IACUC committee, prior to testing in vivo. With these tests ready to graph, we hope we can obtain an approved protocol to commence the in vivo studies in the near future.
Figure 1. Assessment of circulating levels of TIMP-4 in human plasma samples. Using known amounts of recombinant human TIMP-4 (R&D Systems) in a commercial ELISA [A] (R&D Systems) we were able to determine the levels of TIMP-4 in patients from LMC. The known amounts of TIMP-4 were used to generate a standard curve [B]. The TIMP-4 levels in samples from consented patients were interpolated using the standard curve and used to generate bar graphs. All measurements were performed in triplicate.
Figure 2. Circulating levels of TIMP-4 in plasma from breast cancer patients. Patients undergoing adjuvant chemotherapy as part of breast cancer treatment were tested for TIMP-4 levels in their blood plasma using a double-sandwich ELISA. Shown in the left panel Pt’s A-C, followed from time of surgery, indicate that the elevated levels of TIMP-4 observed in breast cancer patients, as compared to healthy control individuals, are not dependent on tumor burden. The early data suggests that treating patients with 6 cycles possibly might be more effective in reducing circulating levels of TIMP-4 than 4 cycles. The right hand panel shows the levels in patients from before starting chemo through treatment cycles and follow-up visits. All bars are average of 3 measurements.
Pre = at time of surgery; co = before starting chemo; c1 – c6 = cycles 1 through 6; F/U 3 = follow-up visit 3 months after completing chemo; F/U 5 = follow-up visit 5 months after completing chemo.
Figure 3. Growth response to PI3K-inhibitor GDC-0941 and TC in human breast cancer cells in the presence or absence of elevated TIMP-4 levels. MDA-MB-468 showed a stronger growth induction in the presence of TIMP-4 than MDA-MB-435S. Both cell lines responded in a similar manner to GDC-0941 and TC a single therapy and in combination. Data points represent average ±SDEV. N=3.