**Determination of a possible mechanism for acquiring chemotherapy resistance**

**Part I: Introduction**

Cancer is a debilitating disease that affects more than one million people annually and is responsible for 22.8%, 575000, deaths in the United States, the second leading cause of death after heart disease [1]. 1 in 2 men and 1 in 3 women will be diagnosed with cancer in their lifetime, and cancer will cost the United States more than 216.6 billion dollars [18,19]. Cancer is characterized by the unregulated growth and proliferation of cells, often accompanied with invasion into the surrounding tissue and spreading to other parts of the body, metastasis. This process is caused by changes in the underlying biology that includes inactivation or suppression of anti-growth, or tumor suppressor, genes as well as over activation of pro-growth genes, ontogenesis [3].

Cancer is treated in several ways which can be broken down into three main categories, chemical therapy, surgery and radiation. For benign cancers, cancers that have not spread to other parts of the body, there is a possibility of cure with surgery and radiation. However, in the case of spreading to other body sites, chemical therapy is the only treatment option that targets all of the cancer cells. Chemical therapy can be further into chemotherapy and targeted therapy. Chemotherapy is the use of cytotoxic agents to target cancer cells [2]. The primary drawback of chemotherapies is that they are non-specific in that they target both normal, healthy cells as well as cancer cells [2]. This results in damage to all rapidly dividing tissues, such as gastric epithelium and lymphoid tissues, as well as cancer cells.

Targeted therapies are the second branch of chemical treatments for cancer. Target therapies, as the name implies, target the underlying molecular biology of cancer cells. Targeted therapies are sometimes coupled with chemotherapies, as some targeted therapies in of themselves do not kill cancer cells; rather they make them far more vulnerable to death [4]. Target therapies often affect either pro-growth cellular cascades or things that are by themselves not pro-growth but are required to maintain the malignant phenotype. While there are many pro-growth and pro survival pathways, a given cancer is usually dependant on a single pathway, a phenomenon known as oncogene addiction [14]. This makes for a number of opportunities in drug development as inhibition of these pathways often results in an extreme susceptibility to and often death of the cancer cells.

As much as treatment for cancer has advanced in the last several decades, drug resistance remains a big problem facing clinicians today [17]. Cancer cells have very unstable genomes, and coupled with their very rapid growth and proliferation give them a very high mutation rate [5]. Tumors are also heterogeneous, meaning that the individual cells that make them up have somewhat dissimilar genomes, and they are not all identical [10]. This results in only the most well adapted cells being selected, meaning that the cells that acquire drug resistance will be selected for making the cancer progressively harder to treat. The mechanism by which cancer cells acquire drug resistance is being extensive and actively researched, and many proteins and mechanisms have been implicates in the process.

One group of proteins implicated in drug resistance is Akt family of proteins, also known as the PKB family [6]. They are a group of serine/threonine kinases that are present in variety of tissues [8]. There are three members in the family Akt1, Akt2, and Akt3 and they mediate many fundamental intracellular signaling pathways such as cell growth, proliferation, protection from apoptosis, modulation of DNA damage genome stability and many more [15,16]. The functions of the Akt family are mediated by growth factors, hormones, cytokines, and nutrient specific receptors [17]. One mechanism for Akt’s involvement in drug resistance is believed to be trough the activation of the PI3K pathway. PI3K, phosphatidylinositol 3 kinase is a protein involved in a immense number cellular processes at a very high level and is typically very closely associated with interpretation of membrane receptor signals[13].

The mechanism for Akt’s involvement in drug resistance is a two –part process, the first of which is mediated by baseline (low) level of Akt/PI3K and DNA damage repair complexes. At a base level of Akt/PI3K there is increased level of cyclin dependant kinase inhibitor 1A, commonly known as p21Cip1, which induces cell cycle arrest. During this arrest damage to the genome from chemotherapeutics is addressed by DNA repair complexes, which moves into the second phase. During part of the process the cancer cell must resume proliferation, thus incremental activation of Akt/PI3k results in ejection of p21Cip1 from the nucleus, resuming proliferation [11,15,16,17]. This is general overview of the mechanisms by which Akt contributes to drug resistance.

The Akt family, as mentioned earlier, consists of 3 members, Akt1, Akt2, and Akt3. The mechanisms that are currently acknowledged do not distinguish much between the different isoforms. Mouse-knockout studies have indicated distinct, yet somewhat overlapping roles for the different isoforms [8]. Akt1 knockouts have shown a decrease in body mass and their cells have shown an increase susceptibility to apoptosis, indication that Akt1 might be involved more closely with apoptosis then either Akt2 or Akt3[8]. Akt2 knockout mice have show type-2 diabetes like symptoms, indicating that Akt2 might play a role in glucose homeostasis [17]. Akt3 knockout mice have shown a significant decrease in brain mass, indicating that Akt3 might be involved in brain development [17]. However, while they may play partly-distinct roles in normal physiology, in cancers overall there is a change in the expression of the different isoforms. For instance, Akt2 has been shown to be amplified in ovarian and pancreatic cancers, Akt1 has been shown to be amplified in gastric cancers, and Akt3 has been shown to be amplified in melanoma and estrogen-receptor negative breast cancers [17]. These are just a few examples where the Akt family proteins are dysregulated in cancers.

Here we wish to examine the effect of isoforms specific inhibition and its impacts on drug resistance in a number of different scenarios’. More specifically we wish to see whether a constitutively active Akt3 pathway correlates with increased drug resistance.

**Part II: Experiment**

The goal of this experiment is to determine whether or not increased baseline levels of Akt3 (constituently active Akt3) correlates with an increase in drug resistance to cis-diamminedichloroplatinum, or cis-platin [7, 9, 12]. If increased Akt3 does in fact lead to increased drug resistance, then I would expect that the cells with higher Akt3 expression to have a higher IC50, the concentration at which half of the cells are killed. I would also expect that inhibition of Akt translation by short hairpin RNA’s would lead to greater drug sensitivity in cells with constitutively active Akt3.

IIA: Cell Lines

Two primary kinds of cell lines are needed for this study, cell line with constitutively active Akt3 and cell lines that do not have constitutively active Akt3. For each category of cell line it would be best to obtain cell line that fit the Akt3 expression criteria for several different cancers, as testing the hypothesis in several distinct cell lines provides an advantage over testing it in a singular set of cell lines.

Using the Cancer Cell Line Encyclopedia (CCLE) database, a few sample cell lines have been chosen that fit the above criteria. The cell lines identified are the EFM192A and MDAMB231 breast cancer cell lines, the NCIH211 and the CALU6 lung cancer cell lines, and the COL0741 and CJM skin cancer cell lines. While this is a preliminary screening and more factors might need to be tested later on, currently these are sufficient to perform the study with. The idea behind using multiple cell lines is that if they all exhibit the results that we expect then we can attribute the results to Akt3, rather than have the results being attributable to second process.

IIB: Study Design

The goal of the experiment is to examine the relationship between Akt3 levels and cis-platin sensitivity and to that end cell culture will be treated with a variety of different combination of cis-platin and shRNA that inhibits Akt3 (this will be designated as shAkt3 henceforth). In order to establish reference sensitivity, each cell line is to be treated with cis-platin exclusively with varying concentrations until the IC50 is determined. Once the reference sensitivity has been established for all cell lines involved the time required to achieve resistance to cis-platin needs to be measured. This can be done by treating the cells with a concentration of cis-plating not enough to kill the entire population, such that growth of the non-killed cells in cis-platin will eventually result in resistance to cis-platin. Resistance will be determined by taking samples and treating them with cis-platin; when the cells exhibit little to no response to cis-platin we can conclude that they are resistant. Once the time to acquire resistance to cis-platin is established, non-resistant cells can treated with cis-platin (at the same concentration used to determine sensitivity) in conjunction with shAkt3 in order to measure the time required to achieve resistance with inhibited Akt3. Finally the IC50 of cells treated with both cis-platin and shAkt3 need to be determined to assess the direct effect of Akt3 expression on drug sensitivity.

**III: Discussion**

Within the hypothesis there exists some information by with different scenario’s can be outlined that would give some ideas as to the results of the experiment. If all goes as expected and Akt3 is responsible for some mechanism for drug resistance, then we would expect that cell lines with higher levels of Akt3 would show lower times to acquire resistance in the presence of cis-platin but longer times to acquire resistance when subjected to both cis-platin and shAkt3. This is due to a suspected dependency on Akt3 for apoptotic protection, i.e. I believe that cell lines that have constitutively higher expression of Akt3 will show oncogenic addiction to Akt3. With relation to the IC50, it is believed that they will have a higher IC50 when exposed to cis-platin alone when compared to the reference and the same or lower of an IC50 when exposed to cis-platin and shAkt3. Again oncogenic addiction might play a role here.

For the cell lines that have low baseline expression of Akt3, it is suspected that they will show little or no change in the time needed o acquire resistance between the cis-platin and the cis-platin+shAkt3. It is also expected that they will show little or moderate change in sensitivity between treatment with cis-platin and treatment with cis-platin+shAkt3.

One major drawback of this experiment is that it cannot be said with the current study design whether or not Akt3 itself is directly responsible for the drug resistance with great certainty. The current design does provide a great deal of information regarding the differences between two groups of Akt3 expression distinct cell lines. One proposition for future studies is to conduct quantitative proteomic examination of the different Akt isoforms (perhaps quantitative western blot) and measuring the change in apoptosis by looking at caspase degradation, a common marker of apoptosis. Changes in apoptosis levels would provide another measure of the efficacy of Akt inhibition as a mechanism for sensitization of cells.

Another drawback is that cancer cells are quickly evolving and may acquire resistance through another mechanism before attempting to acquire resistance through Akt, thus influencing the time required to achieve resistance, as well as the drug sensitivity. This can be addressed by using more specific measures of cellular activities, such as measurement of Akt3 levels intracellular, however that is outside the scope of this study. Overall, despite the drawbacks, this study provides an excellent opportunity to assess the effect of Akt3 on drug resistance and lays the groundwork for future studies to expand on this design and test more specific details in order to full elucidate the nature of drug resistance acquired through Akt.

**References**

1. American Cancer Society. (2014). Cancer Facts & Figures. *Cancer Facts and Figures*. Retrieved from http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf
2. Cepeda, V., Fuertes, M. A., Castilla, J., Alonso, C., Quevedo, C., & Pérez, J. M. (2007). Biochemical mechanisms of cisplatin cytotoxicity. *Anti-Cancer Agents in Medicinal Chemistry*, *7*, 3–18. doi:10.2174/187152007779314044
3. Chaffer, C. L., & Weinberg, R. A. (2011). A perspective on cancer cell metastasis. *Science (New York, N.Y.)*, *331*, 1559–1564. doi:10.1126/science.1203543
4. Dannenberg Jan-Hermen, J. H., & Berns, A. (2010). Drugging Drug Resistance. *Cell*. doi:10.1016/j.cell.2010.03.020
5. Felsher, D. W. (2008). Tumor dormancy and oncogene addiction. *APMIS*. doi:10.1111/j.1600-0463.2008.01037.x
6. Franke, T. F. (2008). PI3K/Akt: getting it right matters. *Oncogene*, *27*, 6473–6488. doi:10.1038/onc.2008.313
7. Galluzzi, L., Senovilla, L., Vitale, I., Michels, J., Martins, I., Kepp, O., … Kroemer, G. (2012). Molecular mechanisms of cisplatin resistance. *Oncogene*. doi:10.1038/onc.2011.384
8. Gonzalez, E., & McGraw, T. E. (2009). The Akt kinases: Isoform specificity in metabolism and cancer. *Cell Cycle*. doi:10.4161/cc.8.16.9335
9. Gottesman, M. M. (2002). Mechanisms of cancer drug resistance. *Annual Review of Medicine*, *53*, 615–627. doi:10.1146/annurev.med.53.082901.103929
10. Greaves, M., & Maley, C. C. (2012). Clonal evolution in cancer. *Nature*. doi:10.1038/nature10762
11. Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., & Mills, G. B. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nature Reviews. Drug Discovery*, *4*, 988–1004. doi:10.1038/nrd1902
12. Longley, D. B., & Johnston, P. G. (2005). Molecular mechanisms of drug resistance. *The Journal of Pathology*, *205*, 275–292. doi:10.1002/path.1706
13. Manning, B. D., & Cantley, L. C. (2007). AKT/PKB Signaling: Navigating Downstream. *Cell*. doi:10.1016/j.cell.2007.06.009
14. McCormick, F. (2011). Cancer therapy based on oncogene addiction. *Journal of Surgical Oncology*. doi:10.1002/jso.21749
15. Mitsuuchi, Y., Johnson, S. W., Selvakumaran, M., Williams, S. J., Hamilton, T. C., & Testa, J. R. (2000). The phosphatidylinositol 3-kinase/AKT signal transduction pathway plays a critical role in the expression of p21(WAF1/CIP1/SDI1) induced by cisplatin and paclitaxel. *Cancer Research*, *60*, 5390–5394.
16. Osaki, M., Oshimura, M., & Ito, H. (2004). PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis : An International Journal on Programmed Cell Death*, *9*, 667–676. doi:10.1023/B:APPT.0000045801.15585.dd
17. Romano, G. (2013). The Role of the Dysfunctional Akt-Related Pathway in Cancer: Establishment and Maintenance of a Malignant Cell Phenotype, Resistance to Therapy, and Future Strategies for Drug Development. *Scientifica*, *2013*, 317186. doi:10.1155/2013/317186
18. Siegel, R., Ma, J., Zou, Z., & Jemal, A. (2014). Cancer statistics, 2014. *CA: A Cancer Journal for Clinicians*, *64*, 9–29. doi:10.3322/caac.21208
19. Siegel, R., Naishadham, D., & Jemal, A. (2013). Cancer statistics, 2013. *CA: A Cancer Journal for Clinicians*, *63*, 11–30. doi:10.3322/caac.21166
20. Vivanco, I., & Sawyers, C. L. (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nature Reviews. Cancer*, *2*, 489–501. doi:10.1038/nrc839
21. Wang, R., & Brattain, M. G. (2006). AKT can be activated in the nucleus. *Cellular Signalling*, *18*, 1722–1731. doi:10.1016/j.cellsig.2006.01.020
22. Xu, N., Lao, Y., Zhang, Y., & Gillespie, D. A. (2012). Akt: A double-edged sword in cell proliferation and genome stability. *Journal of Oncology*. doi:10.1155/2012/951724