Nathan Schnaper Summer Intern Program in Cancer Research
2013 Symposium
Intern Abstracts and Presentations

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-AND-
Schnaper mentors and their lab members!

2013 Intern Presentation Schedule

Time	Intern/ Presentation Title	Mentor
9:00-9:20	Brandon Khor, Role of RNase-L on Intestinal Epithelial Cell Differentiation	Bret Hassel
9:25-9:50	Catherine Landis, The role and regulation of tristetraprolin in breast cancer	Gerald Wilson
9:55-10:15	Alise Moseson, Plasminogen Activator Inhibitor Type 2 Mediated Protection against Calcimycin-Induced Cell Death	Toni Antalis
10:20-10:40	Adip Bhargav, Crystal Structure Determination of Human Quinolinic Acid Phosphoribosyltransferase Complexes	Eric Toth
10:45-11:05	Pragyashree Sharma Basyal, The potential role of exosome in breast cancer.	Austin Yang
11:10-11:30	Zach Stocksdale, ERK activity mediates cancer metastasis	Paul Shapiro
11:30-12:30	LUNCH BREAK	
12:35-12:55	Mario Tarasco, miRNA Regulation of Double Strand Break DNA repair	Kara Scheibner
1:00-1:20	Rimsha Jalees Afzal, Promotion of MicroRNA-34a Expression with RPL-22 Interaction in a Yeast Three Hybrid Screen	Tami Kingsbury
1:25-1:45	Matthew Fowler, Effects of Trastuzumab on RUNX2 positive cells	Tony Passaniti
1:50-2:10	Omasiri Wonodi, Cancer Therapeutics: The Effect of Dimeric Napthoquinones in Acute Myeloid Leukemia	Rena Lapidus
2:15-2:35	Brendan King, Is Plasma Membrane-Bound Fn14 a Substrate for Proteolytic Processing?	Jeff Winkles
2:40-3:00	Beatriz Tenorio, Looking at the FACS: Which drugs are best for cancer therapy?	Eduardo Davila
3:05-3:25	Matthew Emrick, Cyclin D1 Effects on Expression of Chemokine Receptors and Ligands	Dhan Kalvakolanu
3:05-3:25	Kanav Markan, Exploring the Roles of ROS in Life and Death Decisions in Lymphoma	Greg Carey

Brandon Khor, St. Olaf University
Mentors: Dr. Bret Hassel, Tiha Long

Role of RNase-L in Intestinal Epithelial Cell Differentiation

Colorectal cancer (CRC) is the second leading cause of cancer related death in the United States, with approximately 143,000 diagnoses and 51,000 deaths annually. The colon is lined with intestinal epithelial cells (IECs) that mature from pluripotent stem cells to differentiated cells in several days. Genetic alterations to IECs can cause defects in cell turnover and differentiation, which can induce carcinogenesis. The endoribonuclease, RNase-L, regulates mRNA transcripts involved in differentiation and cell cycle regulation. Previous work from our lab revealed that RNase-L deficiency increases tumor burden in a murine model of colitis-associated CRC. Therefore, we hypothesize that RNase-L acts as a tumor suppressor by promoting differentiation in IECs, which is associated with exit from the cell cycle and decreased proliferation. In order to test this hypothesis, we overexpressed RNase-L in the CRC cell lines, Caco-2 and HCT116, and investigated the impact on markers of differentiation and proliferation. Consistent with our hypothesis, we discovered that RNase-L protein levels increase during the differentiation of IEC-like Caco-2 cells and decrease following terminal differentiation. Many proteins, including villin and occludin, increase as IECs differentiate and can be used as markers of this process. Following transfection, we used western blotting to determine if RNase-L overexpression increases the levels of differentiation. While ectopic RNase-L expression did not affect occludin in either cell line, villin was increased following transfection of RNase-L into the HCT 116 cells. Next, we performed a proliferation assay to determine if RNase-L overexpression inhibits proliferation of IECs. RNase-L transfection did not alter cell proliferation which may reflect the low transfection efficiency obtained for these cell lines. Overall, this study demonstrates that RNase-L increases during IEC differentiation and induces the expression of the differentiation marker, villin. Further studies will measure the impact of RNase-L modulation on other markers of differentiation and cell cycle proteins.

Catherine Landis, Towson University
Mentor: Dr. Gerald Wilson

The role and regulation of tristetraprolin in breast cancer

The rate of mRNA decay plays an important role in the regulation of gene expression, which is often negatively altered in cancers; the cell is able to modulate mRNA stability through the activity of AU-rich elements (AREs), which are located in the 3' untranslated region. Proteins that recognize and bind to AREs are able to control the rate of mRNA decay and thus influence tumorigenic phenotypes; one such protein is tristetraprolin (TTP). In breast cancer patients, as tumor grade increases TTP expression decreases. We have been exploring the transcriptional silencing of TTP in high grade breast cancer and the role of TTP as a tumor-suppressor.

In the metastatic breast cancer cell line MDA-MB-231 we have been able to induce TTP expression with TPA, which activates all MAPK pathways through activation of protein kinase C. Furthermore, qRT-PCR and western blots have shown that TTP mRNA levels increase when ERK is un-phosphorylated, and therefore inactive. ERK is an important MAPK protein that is constitutively active in this cell line. This reveals an interesting relationship between ERK deactivation and TTP transcription in metastatic breast cancer that is previously unreported.

We have also focused on TTP's tumor-suppressor role. Preliminary experiments have shown that upon TTP expression the rate of cell cycle progression and proliferation in MDA-MB231 cells is reduced. The protein and mRNA levels of G1/S regulators like cyclin D1 and E are reduced upon TTP expression, and cMYC appears to be post-translationaly modified. Preliminary ActD time courses reveal little change in mRNA half-life for cell cycle regulators, implying that TTP may act on a proliferation master regulator. Finally, MDA-MB-123 cells expressing undergo morphological changes consistent with increased differentiation. A look at TWIST mRNA levels, an established regulator of EMT, revealed that upon TTP expression TWIST levels are reduced to those seen in MCF7 cells.

Alise Moseson, University of Maryland
Mentor: Dr. Toni Antalis

Plasminogen Activator Inhibitor Type 2 Mediated Protection against Calcimycin-Induced Cell Death

Plasminogen activator inhibitor type 2 (PAI-2) is a serine protease inhibitor (SERPIN) that is deregulated in cancer and is produced in response to cellular stress. Specifically, PAI-2 has been reported to protect against cell death induced by TNFα, LPS, and alphaviruses – all which lead to an increase in intracellular calcium. Intracellular calcium homeostasis is critical for proper cellular function and its deregulation leads to cell death. Recent studies in our lab have shown that PAI-2 protects against cell death induced by the calcium ionophore Calcimycin (A23187). Calcimycin is a divalent cation carrier that is known to cause cell death by dramatically increasing intracellular calcium. Given these findings, we are investigating the mechanism of PAI-2-mediated cytoprotection against Calcimycin-induced cell death. Utilizing an ovarian cancer cell line model stably expressing wildtype PAI-2 or an empty vector, we find that PAI-2 expression protects from Calcimycin-induced cell death as measured by MTT cell viability assay and reduced Poly (ADP)-ribose polymerase (PARP) cleavage. To test whether PAI-2 requires its catalytic or protein binding activity, we utilized an ovarian cancer cell line model stably expressing wildtype or mutant forms of PAI-2 and performed a MTT cell viability assay. Our preliminary findings suggest that PAI-2's protein binding domain, the CD loop, is most critical for protection against Calcimycin-induced cell death. There are several proteins that bind the CD loop which may be involved in PAI-2 mediated cytoprotection. Taken together, these data provide new insight into the mechanism of PAI-2 cytoprotective activity.

Adip G. Bhargav, University of Maryland
Mentor: Dr. Eric Toth

Crystal Structure Determination of Human Quinolinic Acid Phosphoribosyltransferase Complexes

Quinolinic Acid Phosphoribosyltransferase (QPRT) is a key enzyme in the kynurenine pathway of tryptophan degradation and the de novo NAD+ biosynthesis pathway. QPRT catabolizes Quinolinic Acid (QUIN) into NAD+ along its reaction pathway, and sits at a critical point in both the de novo synthesis pathway and the NAD+ salvage pathway. QUIN is also a neurodestructive metabolite that is cited in various neurodegenerative diseases. Furthermore, gliomas undergo a metabolic shift that makes them dependent on QUIN as a precursor for NAD+. Therefore, modulation of QPRT activity has the potential to ameliorate aspects of various disease states including cancer. Finally, QUIN accumulation in gliomas also has been associated with a malignant phenotype. Better understanding of the binding sites, structure, and function of QPRT is essential for future drug design and development. Here we report the initial crystallization attempts of two enzyme-substrate complexes along the reaction pathway of QPRT. First, we crystallized QUIN and Phosphoribosyl Pyrophosphate (PRPP) bound to QPRT which is a pre-catalytic state. Metals, known to inhibit catalysis were used in this case to attempt to trap the complex in the desired state. Second, we crystallized Nicotinic Acid Mononucleotide (NAMN) and Pyrophosphate (PPi) bound to QPRT which is an intermediate along the pathway. Crystals were obtained in both cases but further optimization is required. The crystals will undergo x-ray diffraction analysis. Then by elucidating the structures of the QPRT intermediates, we will look for possible inhibitors and activators based on the structural data and high-throughput screening which will be conducted in the near future. Enzymatic assays with the drugs will be performed to confirm their effects after which the ultimate goal will be therapeutic drug development via various model systems and ultimately clinical trials.

Pragyashree Sharma Basyal, CCBC
Mentor: Austin Yang

The potential role of exosome in breast cancer.

Breast cancer is the most common cancer in women. Exosomes are micro vesicles, 30-100nm in diameter. They are released into the extracellular environment from a variety of different cells like tumor cells, dendritic cells, lymphoid cells, mesothelial cells, epithelial cells, or cells from different tissues or organs. In cancer, exosomes have also been found to be secreted by various types of tumors. It has been suggested to play many critical roles in cell-cell communication and the regulation of cancer metastasis. VPS4B is an AAA ATPase, a protein complex associated with disassembly of the ESCRT-III, it reduces the efficiency of degradation of protein. It also helps in endosomal sorting complexes required for transport and energy too. It participates in vesicular trafficking and autophagosome in mammalian cells. Recently, it was reported that ablation of VPS4 and MVB dysfunction often occur in high grade and recurrent breast cancer. Therefore, the goal of this study is to use a comparative proteomic approach to understand the molecular machinery involved in the formation of Multi Vesicle Body-dependent exosome secretion. Results from preliminary studies suggest that manipulation of VPS4B can abnormally activate and alter the content of secreted exosomes, which ultimately leads to the resistance of breast cancer cells to various chemotherapeutic agents.

Zach Stocksdale, Washington College
Mentor: Paul Shapiro

ERK activity mediates cancer metastasis.

Extracellular signal-regulated kinases (ERK) mediate several cell processes including proliferation, survival, differentiation, and migration. Constitutive activation of ERK proteins has been implicated in the development and progression of many human cancers. Therapeutic inhibition of cell migratory function by targeting ERK phosphorylation sites is desirable, as metastases are the leading cause of death from cancer. Here we report SH3BP1, an exocyst-associated RhoGAP, as a novel ERK phosphorylation site. SH3BP1 has been implicated in cell motility and is known to be heavily phosphorylated in breast cancer cells resistant to chemotherapy. Western blot analyses were performed using three cancer cell lines transfected with a wild type SH3BP1 construct. SKBR3 VPS4B knock downs (drug-resistant breast cancer cells) were treated with AZD6244 (MEK1/2 inhibitor) and ERKi (ERK inhibitor), followed by EGF stimulation. A375 cells (melanoma cancer cell line with $BRAF^{V600E}$ mutation) were treated with U0126, a different MEK1/2 inhibitor, and with ERKi. Both tests yielded inhibition of SH3BP1 phosphorylation events. HeLa cells were also transfected with WT or constitutively active MEK1 to support that ERK activity was driving SH3BP1 phosphorylation, as EGF can stimulate a variety of signaling pathways. Cells transfected with constitutively active MEK1 showed an increase in SH3BP1 phosphorylation. These results demonstrate that the ERK signaling pathway drives SH3BP1 phosphorylation. Future studies will aim to identify the location of specific ERK phosphorylation sites on SH3BP1, to create mutant constructs, and to examine their phenotype. We predict that the SH3BP1 mutant cells would be unable to migrate effectively.

Mario Tarasco, Monroe College
Mentor: Kara Scheibner

miRNA Regulation of Double Strand Break DNA repair

Leukemia is a cancer of the blood originating in the bone marrow characterized by abnormal growth of leukocytes that crowd out normal cells. Many leukemias are successfully treated with a class of drugs known as Tyrosine Kinase Inhibitors (TKI) and have vastly improved survival rates. Unfortunately, some leukemias have developed drug resistance which has prompted the search for new and novel ways to combat this disease. Principal to the development of drug resistance are acquired mutations. Numerous factors are implicated in causing and/or exacerbating these mutations including DNA Double Strand Breaks (DSB) and incorrect repair of said DSBs.

microRNAs are an important class of small, non-coding RNAs that are known to prevent protein synthesis and many are known to be either tumor suppressors or oncomirs. DSBs are repaired through two major pathways: Homologous Recombination (HR) and Non-Homologous End-Joining (NHEJ). NHEJ is a highly error prone repair pathway that is utilized by cells to repair DSBs. Alt-NHEJ is an even more error prone pathway that drives DSB repair and is implicated in causing additional mutations in cancer cells. Two important proteins, PARP1 and LIG3 are known to drive the Alt-NHEJ pathway. Evidence suggests that some tumor suppressor miRs may regulate the expression of PARP1 and LIG3. We investigated three miRs, miR-34a, miR-22 and miR-150, for their potential to knockdown PARP1 and LIG3 respectively. Experiments were carried out by transfecting K562 cells (CML cells in blast crisis) with the miR mimics, harvesting protein and running a western on said protein. Results showed a significant knockdown in both proteins by all three miRs at various timecourses. Future directions are to repeat the experiments, verify predicted binding sites for the studied miRs and to develop potential novel treatments that can inhibit PARP1/LIG3.

Rimsha Jalees Afzal, Meredith College
Mentor: Tami Kingsbury

Promotion of MicroRNA-34a Expression with RPL-22 Interaction in a Yeast Three Hybrid Screen

The basics of this experiment stem from understanding the mechanism behind microRNA processing and functionality; in specific, microRNA-34a (miR-34a). MiR-34a is a master tumor suppressor that inhibits metastatic growth by repressing specific genes involved in cancer signaling pathways. MiR-34a is also deregulated in several different types of cancers. Based on what is understood from miRNA processing, the target is to identify novel regulators of miR-34a processing. In order to approach this, a Yeast Three Hybrid Screen was conducted. This is a useful system that allows one to analyze and detect detailed interactions between RNA and protein. The aim is to test for candidates that might promote miR-34a expression. Candidates for screening were obtained from a cDNA library originated from a human fetal brain. A cDNA library generates a collection of constructs that are further tested in comprehensive screen for possible miR-34a interaction. The screening strategy included picking candidate colonies, isolating library plasmids, yeast transformations to retest certain candidates, and sequencing these plasmids. RPL-22 (Ribosomal protein 22) served as the main candidate for this screen; inactivation of the RPL-22 gene leads to cell transformation. In order to validate that RPL-22 positively interacted with miR-34a, three different strategies were conducted: testing antibody to detect endogenous protein against RPL-22, siRNA knockout of RPL-22, and an immunoprecipitation binding assay. RPL-22 and control antibodies worked for the overexpression construct in order to further test the interaction in human cells. These studies are relevant to future oncogenic research in the sense that RNA binding proteins will serve as novel therapeutic targets for cancer which may or may not be regulated by specific microRNAs.

Matthew Fowler, St. Mary's College of Maryland
Mentor: Antonino Passaniti

Effects of Trastuzumab on RUNX2 positive cells

RUNX2, a transcription factor, in normally functioning cells helps promote osteogenesis. RUNX2, however, has also been implicated in the promotion of cancer progression. Constitutive activation of signaling pathways in tumor cells results in abnormal expression and activation of RUNX2. HER2-overexpressing breast cancers often show significantly increased activation of the HER2 signaling pathway. There is a positive correlation between RUNX2-positive cells and the expresion of HER2. Herceptin (Trastuzamab) is an antibody that interferes with the HER2/neu receptor and inhibits the proliferation of breast cancer cells. In this study, we investigated whether RUNX2-positive MCF7 breast cancer cells with upregulated HER2 expression would respond to Herceptin. We found that Herceptin inhibited RUNX2-positive MCF7 colony formation in suspension. Further studies may elucidate the mechanisms by which RUNX2 activates HER2 expression and whether Herceptin may be an effective treatment for RUNX2-positive tumors.

Omasiri Wonodi, University of Maryland
Mentor: Rena Lapidus

Cancer Therapeutics: The Effect of Dimeric Napthoquinones in Acute Myeloid Leukemia

Acute myeloid leukemia (AML), a blood cancer, is the most common type of acute leukemia found in adults. This study assessed the anti-cancer properties of a novel drug family, dimeric napthoquinones (BiQ), in AML. BiQs are unique molecules that have been shown to induce cytoxicity in human prostate cancer cells by inducing reactive oxygen species (ROS). Through cyclic voltammetry studies, it has been demonstrated that BiQs are able to undergo four redox steps, inducing the initiation and propagation of free radical chain reactions to produce ROS. In this study, we tested these compounds in two AML cell lines, MOLM-14 (FLT3-ITD mutant) and THP-1 (FLT3-wild-type and two primary AML cancer cells, AML-15 and AML-16. We hypothesize that treating FLT3-ITD AML cells will produce insurmountable levels of ROS and DNA damage, inducing apoptosis in AML cells, including those with FLT3-ITD, and to a lesser extent the FLT3 WT AML cells. The MOLM-14 and THP-1 cells were treated with a dose response of three different derivatives of BiQs, B1a, E6a, and KS (control) for 72 hours. Endpoints included alamar blue proliferation assay and trypan blue exclusion (viability). The IC50 values generated for both cells lines were in the single digit uM range. To see how the BiQs would affect two primary cell lines, AML-15 and AML-16, we generated IC50 values and tested viability after 48 hours of exposure. In the future, we intend to test the effect of these compounds on normal cells and to evaluate generation of ROS, and apoptotic markers by Western analysis and flow cytometry in the cancer cells.

Brendan King, University of Virginia
Mentor: Jeff Winkles

Is Plasma Membrane-Bound Fn14 a Substrate for Proteolytic Processing?

The cytokine TNF-like WEAK inducer of apoptosis, or TWEAK, controls many cellular activities including cell growth, survival, and differentiation. TWEAK acts by binding a small cell-surface receptor named Fn14, which is highly expressed in damaged or diseased tissues, including many types of solid tumors. Many of the cellular responses induced by the TWEAK/Fn14 pathway, including angiogenesis and cell invasion, are linked to tumor growth and metastasis; thus, both TWEAK and Fn14 are molecular targets for cancer therapeutics. Fn14 is initially synthesized as a 129-amino-acid protein that is then proteolytically processed by signal peptidase into an ~17-kDa, 102-amino-acid cell-surface receptor. However, Western blots using an antibody specific to the Fn14 cytosolic tail have revealed a smaller molecular weight protein of ~12-kDa that is present in both normal and cancerous cells. We hypothesize that the smaller Fn14 protein is the product of either ectodomain shedding, wherein a single cleavage event results in the release of the receptor's extracellular domain into the media, or regulated intramembrane proteolysis (RIP), wherein two sequential protease cleavage events result in the release of the extracellular domain into the media and the cytosolic domain into the cytosol. To begin to test this hypothesis, we treated human and mouse breast cancer cell lines with various ADAM (a disintegrin and metalloproteinase) and γ-secretase inhibitors. We observed significant changes in the ratio of the amount of full-length and lower molecular weight Fn14 receptor. These results indicate that Fn14 may undergo ectodomain shedding or RIP, but additional experiments are required to confirm these findings and identify the precise Fn14 processing pathway.

Beatriz Tenorio, Mount Saint Mary's College
Dr. Eduardo Davila

Looking at the FACS: Which drugs are best for cancer therapy?

The immune system is composed of a variety of cell types. Amongst these, CD8 T cells are capable of recognizing and destroying tumor cells. However, recent data indicate that the tumors 'fight' back and can express proteins that kill T cells and thereby negate antitumor immune responses. For example, PD-L1 on tumor cells binds to PD-1 receptors expressed on activated T-cells causing T-cell anergy (unresponsiveness) or apoptosis (death). PD-L1 expression in tumor cells has been linked to immune response evasion, suggesting that blocking or decreasing PD-L1 is a viable form of combating cancer. Although there are many studies looking into PD-1 antibodies, blockade for cancer therapies, and making breakthrough drugs to address this, none have conducted a mass screening of already available drugs that may affect current immunotherapy outcome due to PD-1/PD-L1 interaction. Some limitations to antibody-mediated therapy include that high liver toxicity, the inability for Abs to penetrate bulky tumors and the fact that Abs can only recognize one specific protein resulting in the possibility that other immunosuppressive ligand kill T cells. In the current studies we tested the hypothesis that current FDA-approved drugs affected PD-L1 expression in tumor cells. We treated A375 melanoma cells with nearly 1000 FDA-approved drugs and stained them for flow cytometry to observe changes in PD-L1 expression. Our data supported drug-induced changes in PD-L1 expression and alluded to certain pathways that interact with PD-L1 expression. Upon completion of in vitro trials, we intend to transition in vivo tests in mice using drugs that up-regulate and down-regulate PD-L1 on tumor cells. In doing this, drugs that down-regulate PD-L1 can be quickly identified and preferred in cancer immunotherapy while drugs that up-regulate PD-L1 would be noted and avoided in cancer immunotherapies.

Matthew Emrick, Whittier College
Mentor: Dhan Kalvakolanu

Cyclin D1 Effects on Expression of Chemokine Receptors and Ligands

Mitosis is governed by tightly controlled environmental signals. When this signaling is dysregulated, cell reproduction rates increase and initiate tumorigenesis. In the G_1 phase, cell function is geared toward growth and survival through metabolism by presence of phase specific cyclins. Cyclins pair with Cyclin-Dependent Kinases where concentrations of each combination propel the cell cycle through the various phases. The G_1 phase relies on the upregulation of Cyclin D's and their pairing with Cyclin-Dependent Kinases 4 and 6 (CDK4/6). Until recently, D1 has been thought to only enter the nucleus by pairing with CDK4/6 as a part of the Cell Cycle Clock. Outside research now shows that D1 acts as a cotranscription factor. Current work in this lab explores the suspicion that increased D1 levels have a positive correlation to increased chemokine receptor/ligand expression as well as to upregulated STAT3, transcription factor of D1, and downregulated GRIM-19, tumor suppressor and inhibitor of STAT3. The hypothesis is that D1 is CDK-independent and enters the nucleus by unknown mechanism to increase the expression of chemokine receptors and ligands as a cotranscription factor for these chemokine targets. Using two cell lines of Head and Neck cancers (HN6 and HN31), the expression of chemokine receptors and ligands initially show two different responses to D1 independence of CDK4/6 when inhibited by a p16INK4a and by statistical analysis of qRT-PCR results. HN6 responds with little change overall although some receptors and ligands show promise that some D1 interaction with DNA leads to increased expression. HN31 responds with greater overall change to nearly all receptors and ligands that were tested. Since one cell line is impacted greater by CDK inhibition, more near future tests have stemmed from this initial panel to test if D1 has a direct relationship to GRIM-19 using the receptors and ligands that show positive correlation between both tested cell lines and introducing a third with transfected GRIM-19 with point mutations.

Kanav Markan, Georgetown University
Mentor: Greg Carey

Exploring the Roles of ROS in Life and Death Decisions in Lymphoma

B cells receive signals through B Cell Receptors (BCR's). If the signal is very strong, this can lead to apoptotic death of the cell, and if too low, it can lead to anergic death of the cell. IgM is a specific type of BCR, and is triggered by the antibody anti-IgM to send a signal to the cell. Reactive Oxygen species (ROS) can accumulate in the cell when electron carriers in the Electron Transport Chain in mitochondria within the cell react with oxygen at the end of the chain, forming superoxide free radical anions. The enzyme Superoxide Dismutase 1 (SOD1) converts superoxide radicals to Hydrogen Peroxide. We hypothesized that finding a way to block ROS production would protect cells from death. Here we examine how B Cell Receptor stimulation affects ROS responses in the cell, which was done through the use of Western Blotting and Flow Analysis. Using fluorescent labeling markers that such as DCFDA, which measures intracellular Hydrogen Peroxide levels, and DHE, which measures intracellular ROS levels, we determined ROS responses in response to BCR stimulation in IgM-expressing B-cell Lymphoma cells, including WEHI-231 cell lines, WEHI-231 combined with BclXL, and Ramos cell lines. DHE fluoresces red upon reacting with superoxide anions to track them, while DCFDA is a fluorogenic dye that is oxidized in the cell in order to fluoresce. In addition, data suggested that BCR signals have been found to suppress SOD1 expression, resulting in decreased Hydrogen Peroxide levels, which leads to apoptosis. Our results suggest that BCR stimulation causes a peak in ROS levels and a decrease in Hydrogen Peroxide levels, that early BCR signals suppress SOD1 expression, loss of Hydrogen Peroxide can result in apoptosis, and that suppression of PI-3-Kinase by BCR stimulation can suppress or alter metabolism.