SAMUEL WAXMAN CANCER RESEARCH FOUNDATION

Applicant 1 Information

Project Title: Modulating Transcription Repressor Sin3 for Targeted Epigenetic Cancer Therapy.

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SAMUEL WAXMAN CANCER RESEARCH FOUNDATION

Applicant 2 Information

Project Title: Modulating Transcription Repressor Sin3 for Targeted Epigenetic Cancer Therapy.

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Lay Summary

Mortality from breast cancer has recently declined, however, the impact of conventional cytotoxic treatment has contributed little to breast cancer survival rates, particularly in advanced stages of this disease. Recent studies have demonstrated that multiple epigenetic programs creating abnormal gene function contribute to the development of breast cancer. Epigenetics is the study of heritable chromatin traits that are not encoded in the DNA sequence and epigenetic mechanisms are a critical factor in the regulation of many cellular processes, including gene expression and DNA replication. As in the long-established case with gene mutations, epigenetic changes are a driving force in carcinogenesis. In contrast to gene mutations, however, epigenetic modifications can be manipulated by targeting factors that are responsible for their establishment and maintenance. This reversibility of potentially harmful epigenetic changes has made the development of drugs targeting these modifications, so-called epidrugs, an attractive avenue of research in cancer therapeutics.

Triple negative breast cancer (lacking receptors for estrogen, progesterone and Her2) accounts for 10-20% of the overall total of breast cancer cases. This particular type of breast cancer, which generally has a poor prognosis, is more common in young African American and Hispanic women. In triple negative cancer there is a group of epigenetically silenced genes essential for normal growth, function and death control and switching off these genes makes a key contribution to the prognostically unfavorable basal phenotype that characterizes this disease. Thus far, there has been modest clinical response from drugs designed to correct these abnormal epigenetic defects and restore essential gene function in breast cancer. ***We believe that more specific epigenetic targets that contribute to the development of breast cancer, once identified, will lead to novel approaches to the treatment of triple negative breast cancer and potentially other cancers.*** To this end Dr. Zelent in collaboration with Drs. Waxman and Farias (Mount Sinai School of Medicine) designed a targeted disruption of a small group of proteins that contribute to gene silencing in breast cancer using a small decoy, which we call SID, to restore essential gene function. The proposed collaborative multi-disciplinary research (also involving the teams of Waxman and Farias as well as a collaboration with Prof. Alan Ashworth at the Institute of Cancer Research) is to develop small molecule inhibitors with the biological properties of SID and evaluate their therapeutic potential in a large number of breast cancer cell lines. Dr. Ming-Ming Zhou has now developed a number of small molecules that mimic SID action and are being tested both in vitro and in vivo with aim to develop the most promising agents for therapeutic intervention. The basic mechanisms of SID decoy action and small molecular SID mimics are also being studied. Results of these more basic studies should offer new insights into the epigenetic bases of breast cancer development and help in the optimization of small molecular SID decoys for anticancer therapy. Successful SID decoy development will offer novel therapeutic options for triple negative breast cancer.

Technical Summary

Significance

Results from Drs. Zelent's, Waxman's and Farias's studies recently published in *PNAS* demonstrate that a decoy peptide corresponding to Sin3 interaction domain (SID) of MAD causes epigenetic reprogramming of targeted genes, characterized by loss of promoter DNA methylation and a dramatic increase in H3K4 methylation.[1] This leads to a basal to luminal phenotypic transition in triple-negative MDA-MB-231 breast cancer cells that is characterized by the re-expression of key epithelial markers such as ERα and E-cadherin.[1] We anticipate that characterization of the mechanisms/pathways that underlie the activity of SID decoy in triple-negative breast cancer will lead to the identification of further druggable targets and provide rationales for combination treatments that will potentially improve patient outcome in this breast cancer subtype in which survival rates remain unacceptably low. This initial work forms a platform for the identification of specific factors (such as Pf1, for example) that may be primary targets of SID decoys and the development of small molecule mimics of SID decoy using structure-function based computational strategies.

Innovation

In the *PNAS* study Drs. Zelent, Waxman and Farias utilized a SID decoy peptide (and in this research proposal Dr. Zelent will test small molecules developed by Dr. Zhou) that specifically blocks interactions between the PAH2 domain of Sin3 and partner proteins.[1] This strategy of blocking specific interactions between oncoproteins and factors required for their transforming activites has thus far only been employed by several groups in hematological malignancies.[2-6] Interference with recruitment of specific transcriptional repressor complexes by SID decoy leading to epigenetic reprogramming represents a conceptually novel approach to therapy in breast cancer.

Summary of the Collaboration

This SWCRF-funded collaborative grant has enabled the continuation of a long-standing collaboration between the groups of Drs Farias, Waxman and Zelent. This relationship has been particularly successful due to the complementary nature of the expertise that both groups possess: Farias and Waxman have great experience in the field of breast cancer research, the use of animal models and cell-based assays, and Zelent has expertise in biochemical and epigenetic techniques. As mentioned above, this resulted in a publication in PNAS and the collaboration with Prof. Zhou (an expert in structure-based ligand design using combined structural/chemical and molecular biology methods) over the last 12 months has enabled this work to move forward with the development of small-molecule mimetics of SID. Furthermore, in addition to the high degree of complementarity between the three groups involved in this collaborative effort, the different perspectives and expertise available foster a climate of intellectual exchange that is very useful for the research process.

Background

In their recent *PNAS* study, Dr. Zelent, in collaboration with Drs. Waxman and Farias, targeted disruption of interactions of the well-characterized PAH2 domain of the Sin3A/B co-repressor, through which Sin3 binds with high affinity to a limited number of Sin3 interaction domain (SID)-containing transcription factors.[7] Sin3 serves as a multisubunit co-repressor scaffold protein[8,9] that regulates transcription by recruiting chromatin modifiers including JARID1A (KDM5A/RBP2) and JARID1B (KDM5B/PLU-1), demethylases specific for di- and tri-methylated lysine 4 of histone H3 (H3K4$^{me2/me3}$). Recently, H3K4 methylation has been demonstrated to be critical for *de novo* DNA methylation.[10-13]

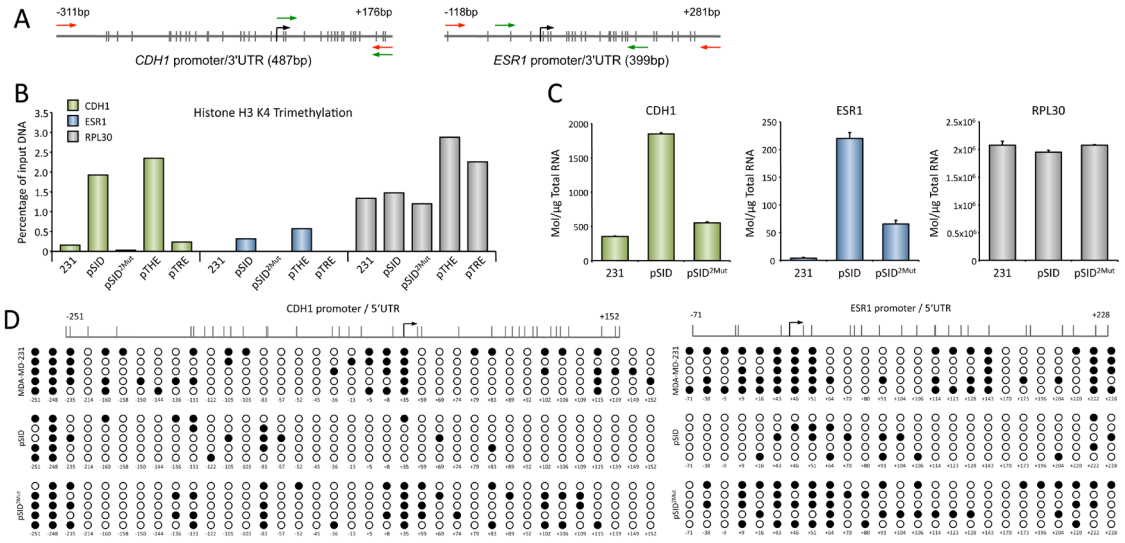

Figure 1. A) *Schematic representations of the promoters and 5' untranslated regions (5'UTR) of CDH1 and ESR1 used in this study.* Positions of CpG dinucleotides are indicated by grey ticks and the transcription initiation sites are represented by black arrows. The relative positions of primer pairs used to PCR amplify immunoprecipitated DNA from ChIP analysis (green arrows) and bisulfite-modified DNA (red arrows) are also indicated. **B)** *H3K4^{me3} levels on the CDH1 and ESR1 promoter regions increase dramatically in response to SID.* ChIP analysis was performed with chromatin from wild type or MDA-MB-231 cells stably expressing FLAG-tagged SID (pSID), mutated SID control (pSID^{2Mut}), a Tet repressor (TetR)-SID fusion, or TetR control. Cross-linked protein-DNA complexes were immunoprecipitated with an anti-trimethyl H3K4 antibody and amplified by real-time PCR. Results are shown as percentage of input DNA. The RPL30 housekeeping gene is shown as a control. **C)** *Real-time PCR analysis of CDH1 and ESR1 gene expression.* Values are shown as molecules per µg total RNA and were derived from the ΔCt between the GAPDH housekeeping gene and the gene of interest. The amount of GAPDH molecules per µg total RNA was determined by absolute quantification. **D)** *The CDH1 and ESR1 promoters undergo demethylation in response to SID.* Following bisulfite modification of wild type or stably transfected MDA-MB-231 cells as indicated, specifically amplified PCR products were sequenced using primers corresponding to the promoter/5'UTR of *ESR1*. Positions of CpG dinucleotides are indicated by grey ticks and the transcription initiation sites are indicated by black arrows. Five clones were sequenced per sample and a black-filled circle represents where a CpG dinucleotide was found to be methylated. Unmethylated CpGs are by represented open circles. CpG positions are shown relative to transcription initiation site.

The *PNAS* study demonstrated that treatment with a 13 amino-acid peptide corresponding to the minimal SID domain (SID peptide), or introduction of a vector encoding the minimal SID sequence, disrupted binding of a prototypic SID-containing transcription factor (i.e. MAD) to the Sin3A/B PAH2 domain.[1] This induced profound changes in morphogenesis, loss of invasive potential and restoration of contact inhibition in mouse and triple-negative human breast cancer models *in vitro* and *in vivo*. This switch from a basal to a more differentiated luminal phenotype was underpinned by epigenetic

reprogramming, contributing to re-expression of E-cadherin, oestrogen receptor α and retinoic acid receptors (*CDH1*, *ESR1* and *RARs*), suggesting reversion of the Epithelial/Mesenchymal Transition (EMT) (Figure 1). Moreover, in human triple-negative cell lines, responsiveness to estrogen, tamoxifen and retinoic acid receptor agonists was restored. Tumor growth was inhibited by 70% when cells obtained from tumours generated in MMTV-myc transgenic models were transfected with SID decoy and inoculated orthotopically in FVB mice. Thus, this study demonstrated that targeted disruption of the interaction between a specific motif of a transcriptional co-repressor and a limited number of partner proteins can induce a differentiated phenotype and produce anti-tumour effects.

Based on our data and through an extensive review of the available literature, we have formulated a candidate mechanism for direct (or indirect) interference by SID decoy with recruitment of the histone demethylases JARID1A and/or JARID1B by specific transcriptional repressors to the promoters of two key breast cancer genes, *CDH1* and *ESR1* (see Figure 2). Experimental evidence indicates that a limited number of factors interact with Sin3 via its PAH2 domain,[7] one of which is a PHD zinc finger protein, Pf1.[13,14] Pf1 has two separate SID that directly bind Sin3, with Pf1SID1 binding PAH2 and Pf1SID2 binding PAH1. Pf1 is part of a Sin3 co-repressor complex also containing JARID1A/1B, EMSY, MRG15 and histone deacetylases that can be recruited by the CtBP co-repressor and epithelial to mesenchymal (EMT) transcription factors such as Slug and Snail in response to EMT-associated Notch signaling.[10,15-17] Of note, expression of E-cadherin blocks Jagged1-mediated Notch signaling in breast cancer cells.[16] Recently, it has also been reported that the Sin3-JARID1A/1B co-repressor complex can be recruited to target genes via interactions between EMSY and Ets-1 (unpublished data, http://www.epigenie.com/Highlighted-Conferences/Abcam-Epigenetics-and-Stem-Cells-2010.html). Ets-1 targets and represses the *CDH1* promoter and is implicated in breast cancer invasion.[18] Furthermore, EMSY is amplified in 13% of sporadic breast cancers[19] and its overexpression has been reported to repress anti-metastatic miR-31 by binding to the promoter of miR-31 via Ets-1 through the ETS binding site (unpublished data, http://www.epigenie.com/Highlighted-Conferences/Abcam-Epigenetics-and-Stem-Cells-2010.html).
These findings suggest that Pf1 plays a key structural role, at least, in the Sin3-JARID1A/1B co-repressor complex that, given our preliminary data, is likely to be implicated in the aberrant epigenetics that underpin transcriptional silencing of epithelial genes in invasive breast cancer.

The promoters of *CDH1* and *ESR1* both contain E-box elements that are directly bound and repressed by a number of EMT-associated transcription factors including Slug, Snail, Twist, and ZEB1 and ZEB2.[20-24] Furthermore, shRNA-mediated knockdown of Snail, Twist or ZEB1 in breast cancer cells, or negative regulation of ZEB1 by miR-200c result in re-expression of E-cadherin and inhibition of breast cancer cell migration and invasion.[22,25-27] Interestingly, knockdown of the co-repressor CtBP, which also directly interacts with Sin3,[28] inhibited hypoxia-induced downregulation of E-cadherin, as well as cancer cell migration and invasion.[29] **Evidence that knockdown of individual components of our model repressor complex leads to re-expression of E-cadherin and loss of invasive potential is consistent with our finding that SID disrupts not only associations mediated directly via PAH2 but may have wider-ranging effects on the integrity of the Sin3 co-repressor complex.**[1] It has recently been demonstrated that migration of prostate cancer cells can switch from restrained to invasive depending on the Eph receptor profile, in particular expression of EphB4 and ephrin-B2.[30] This scenario is replicated in MDA-MB-231 cells and consistent with our model, expression of both EphB4 and ephrin-B2 in ES cells is tightly negatively regulated by E-cadherin.[31]

We hypothesize that SID decoy also "locks in" reactivation of *CDH1*, for example, by indirectly stimulating recruitment of AP-2α/γ and hypophosphorylated Rb to the *CDH1* promoter.[32] Our data show that expression of Cyclin D1 is repressed in response to SID decoy, which is consistent with re-expression of E-cadherin and its role in displacing LEF-1 from a complex with β-catenin that transactivates Cyclin D1.[33,34] A target of Cyclin D1, Rb is abnormally phosphorylated in MDA-MB-231 cells and this process is

likely to lead to its activation.[35] Of note, siRNA-mediated depletion of Rb induces phenotypic changes associated with EMT in ERα/E-cadherin positive MCF7 breast cancer cells[36] while forced expression of AP-2α and AP-2γ inhibited tumorigenicity of MDA-MB-231 cells transplanted in nude mice.[37]

In addition to investigating E-cadherin, we have also focused on ERα as a key target of SID decoy.[1] Expression of ERα is clearly linked to the genetic program specifying the epithelial phenotype of luminal-type breast cancers, which are generally more differentiated and less invasive.[38,39] Re-expression of ERα impacts significantly on our model as it represses EMT-associated transcriptional repressors such as Slug and activates expression of E-cadherin both directly and indirectly.[40,41] A defining feature of EMT is a reduction in E-cadherin levels and, in fact, loss of E-cadherin function is sufficient to induce tumor cell migration and invasion and tumor progression *in vitro* and *in vivo*, and occurs during malignant progression in almost all epithelial cancers, serving as a clinical indicator for poor prognosis and metastasis.[42] In breast cancer, recent research indicates that patients with triple-negative breast cancer are more than twice as likely to overexpress EMT genes compared to non triple-negative patients.[43]

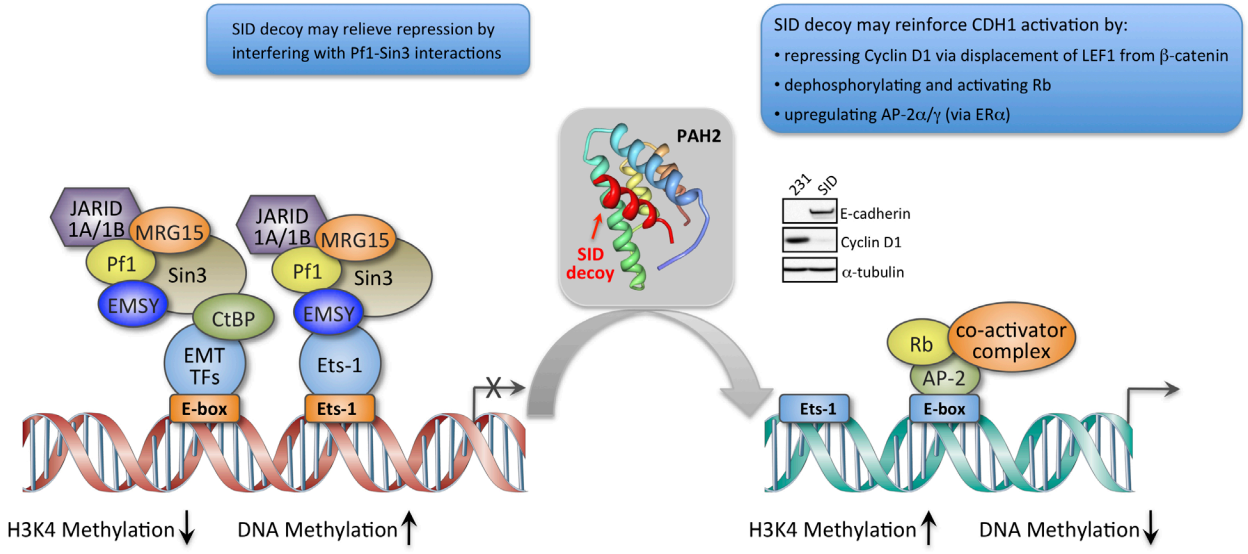

Figure 2. A model for the mechanism underlying the reversal of triple-negative breast cancer basal phenotype in response to SID decoy. Our data suggest that a major component of silencing key epithelial genes such as *CDH1*, depicted here, is Sin3-mediated recruitment of JARID1A/1B H3K4 demethylase activity. Sin3 is itself recruited by EMT-associated transcriptional repressors (TFs) such as ZEB1/2, Snail, Slug and Twist, or Ets-1, as indicated. In our model, treatment with SID decoy interferes with Pf1 interactions with Sin3 via PAH2, leading to destabilization of the repressor complex and loss of H3K4 demethylase activity. Following derepression of *CDH1* through loss of JARID1A/1B recruitment, our model suggests that SID decoy reinforces expression of E-cadherin by causing repression of Cyclin D1, dephosphorylation and activation of Rb, and transcriptional activation of *CDH1* via AP-2 TFs. In the interest of clarity the role of ERα in the positive transcriptional regulation of *CDH1* and repression of EMT-associated TFs has not been presented in this model.

Progress Report – Dr. Arthur Zelent

To determinethe mechanisms of epigenetic reprogramming that underlie the biological/therapeutic effects of SID decoy interference in triple-negative breast cancer models

During the past year of this grant we continued to test *in vitro* by GST pull-down and *in vivo* by DuoLink II assay (Olink Bioscience) candidate SMI of PAH2-SID interaction identified by computational by Ming-Ming Zhou's group. Both *in vitro* and *in vivo* results showed MS6048905 (compound 16 in **FIGURE 3** below) to have the optimal activity. In addition to MAD1 SID this compound was as effective as SID peptide in disrupting Sin3A interaction with Jarid1B and Pf1 proteins. Compound MS6241743 has not been tested yet. Based on its stability, solubility and binding affinity data this compound appears to be most promising and we plan to test it's activity *in vivo* and *in vitro* using the DuoLink II and GST pull down assays. Using the above systems we have also tested a number of modified SID peptides to evaluate whether the effects could be improved due to modification, enhanced stability and/or cell/nuclear membrane permeability. However, the original peptide remained to be most active and we continue using it in all subsequent experiments.

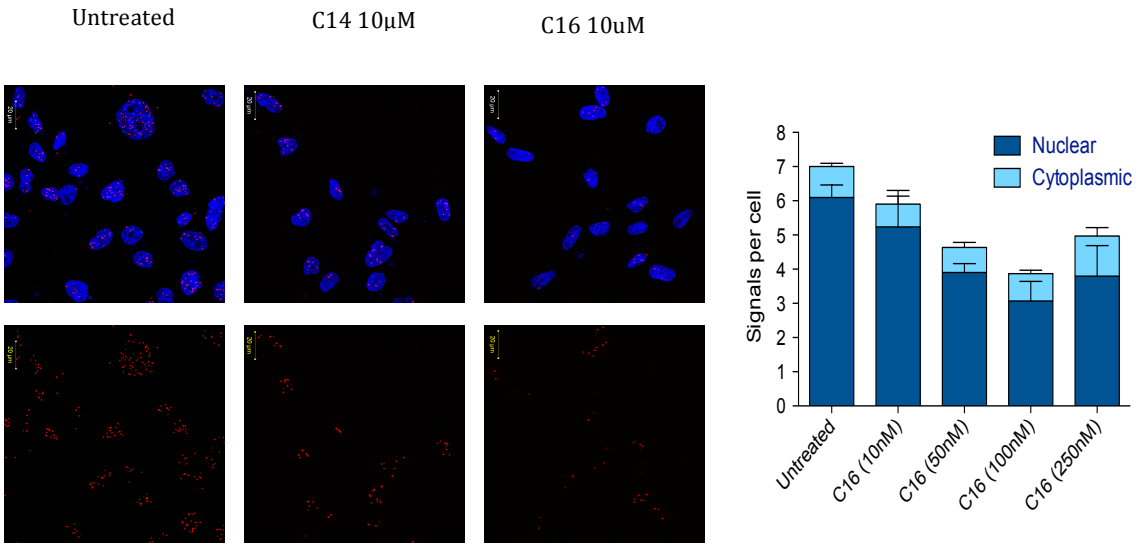

FIGURE 3. Representative images of Sin3A and MAD1 interactions with or without C14 and C16 treatment for 24 hours as indicated. Interactions were visualized with DuoLink assay (Olink Bioscience) according to manufacturer's instructions. Cells were stained with the following antibodies: monoclonal Sin3a (Santa Cruz, sc-5299) and polyclonal Mad (Santa Cruz, sc-222). Images of Sin3A+MAD interactions with or without compound treatment were analyzed with Duolink ImageTool software (Right). Treatments were performed in quadruplicate and errors bars represent standard deviations.

Using the SID peptide we have now carried out gene expression and preliminary ChIP-seq analyses. The ChIP-seq data indicate that both Sin3A and Jarid1B are associated with genomic regions that are consistent with previous reports. We are now carrying on with in depth analysis of the array data and performing ChIP-seq experiments with anti-Sin3A antibody +/- the SID peptide. In order to be able to carry on with ChIP-seq analysis of Jarid1B and Pf1 proteins we needed to purchase custom made/affinity purified antibodies from Eurogentec. Once the ChIP-seq analysis is completed both the gene array expression data and genome wide binding data for the above factors will be correlated. Should RE-ChIP experiments be required we will use a novel technique

developed in the Zelent's lab that eliminates the need for RE-ChIP as only when two target epitopes are in direct proximity can the cross-linked DNA-protein fragments be immunoprecipitated.

TABLE 1. Gene Ontology analysis was carried out on the array data highlighting modulations of genes involved in biological processes such as metabolism, apoptosis, development and growth. Top scoring apoptosis associated genes are listed in the above table. Underlined genes were confirmed with qRT-PCR.

Apoptotic Process	**Enrichment Factor**	**p-value**				
	7.53938	**0.000531728**				
Gene Symbol	**RefSeq**	**p-value(Treatment)**	**Fold-Change(24h vs. control)**	**p-value(24h vs. control)**	**Fold-Change(72h vs. control)**	**p-value(72h vs. control)**
IL24	AY641440	0.000447958	34.5308	0.000285009	24.7599	0.000418654
PIM1	AK301158	0.00436338	4.4072	0.0029271	4.03504	0.0036708
MAGED1	AF124440	0.00252409	3.99873	0.00126724	2.86901	0.00353763
NGF	CR541855	0.000339451	2.68977	0.000128217	1.51686	0.00355565
GSN	AK092797	0.00417218	2.63968	0.00203738	2.04923	0.00618679
KLF11	AF272830	0.0013162	2.4617	0.000754973	2.1451	0.00142373
SRGN	BC015516	0.0456467	2.3596	0.0215077	1.80059	0.066063
DNASE2	AB004574	0.00331352	2.33889	0.00148827	1.77056	0.00644629
TNFSF10	AK296085	0.00534753	2.33304	0.00206824	1.34413	0.0680729
DRAM2	AL833098	0.00107626	2.27697	0.000585081	1.95385	0.00128929
TNFRSF1B	AY148473	0.000595819	2.05693	0.000288638	1.7013	0.000942508
SATB1	ENST00000475083	0.0490115	2.03602	0.0611102	2.63878	0.0243275
FKBP8	AK300571	0.00375472	2.00104	0.00194056	1.71792	0.00486291
LITAF	AB034747	0.00255705	1.95918	0.0012202	1.62626	0.00410376
DFNA5	AF073308	0.00661557	1.95656	0.00447434	1.8883	0.00544831
MAGEH1	AF143235	0.00111686	1.92794	0.00054895	1.63241	0.00168179
MFSD10	BC001502	0.0190456	1.879	0.0102869	1.66455	0.0210328
PPARD	AK296425	0.00437508	1.83199	0.0019824	1.50806	0.0081657

Initial analysis of the gene array data has indicated greatest level of expression changes among genes associated with cell growth, apoptosis and metabolism. Interestingly, among the apoptosis associated genes, the top-scoring gene (and the top scoring gene for the entire analysis) is *IL24*. IL24, also called MDA-7 for melanoma differentiation associated antigen-7, possesses ubiquitous tumor cell pro-apoptotic activity and causes breast cancer cells to arrest in G2/M phase, ultimately leading to apoptosis. List of top scoring apoptosis associated genes is presented in the above table.

In Collaboration with Alan Ashworth and Chris Lord (ICR, London) we have carried out a drug sensitization screen as well siRNAdrug sensitivity scree using primarily compound 16. Both sensitizing and desensitizing hits were identified. Analysis is still in progress but

it is encouraging that C16 (as well as other compounds and peptide) sensitized TNBC cells to tamoxifen effects, which is consistent with our earlier funding. siRNA results on the other hand have indicated both compound specific targets and those common to all compounds. For example ARID1A, a gene encoding chromatin-remodeling protein thus fitting our view of epigenetic mechanisms of action, was a common hit to all the compounds. Further analysis of the siRNA screens and validation experiments are now in progress.

Progress Report – Dr. Ming-Ming Zhou

To Determine the Molecular Basis of Small Molecule Recognition by the Sin3A PAH2 Domain

The goal of our collaborative project is to develop small molecules to modulate the Sin3 co-repressor complex function in epigenetic control of gene transcriptional silencing. In Aim 2, we proposed to conduct target structure-guided computational screening to identify small molecule compounds that bind to the PAH2 domain of Sin3. Our new small molecule compounds are being evaluated *in vitro* for their ability to block Mad Sin3-interaction domain (SID) binding to the PAH2 domain. Further, Our collaborator, Dr. Arthur Zelent is assessing the consequence of chemical disruption of Mad-Sin3 interactions in Sin3 co-repressor function in repression of its target genes (see Aim 1). We expect that the emerging small-molecule mSin3A inhibitors will help validate it as a new therapeutic target for treatment of breast cancer. Below, we provide a brief update on the progress of our study.

(A) Structure-Activity Relationship Study of Lead Compounds – We have discovered several promising small molecule compounds using structure-guided approaches that are capable of inhibiting the mSin3A-PAH2 domain binding to MAD SID peptide. Among them are MS6048905 and MS6241743, as well as selamectin, which show to inhibit Sin3A transcriptional activity in various cell-based experiments (see above Aims 1 and 2). However, unlike the former small molecules, selamectin, a much larger cyclic molecule, has very low solubility in aqueous solution, thus preventing us from determining its binding affinity to mSin3A-PAH2 domain using our fluorescence anisotropy binding assay using a FITC-conjugated SID peptide as the assay probe. In an effort to address this problem, we have conducted extensive solvent optimization and developed a solvent system for NMR binding study that consists of 50 mM Tris-HCl buffer of pH 8.0, 150 mM NcCl and 20% DMSO. Because of high content of DMSO, this solvent system is still not suitable for our fluorescence anisotropy binding assay, but it allowed us to conduct NMR binding study to assess compound binding to the protein. Indeed, we used this NMR method to study mSin3A PAH2 domain binding to ivermectin, a chemical analog of selamectin, which shows slight better solubility in aqueous solution (see **Figure 1**). As shown in 2D NMR HSQC spectra of the ^{15}N-labeled mSin3A PAH2 domain, many NMR resonance signals of the protein exhibited major chemical shift perturbation upon addition of ivermectin,

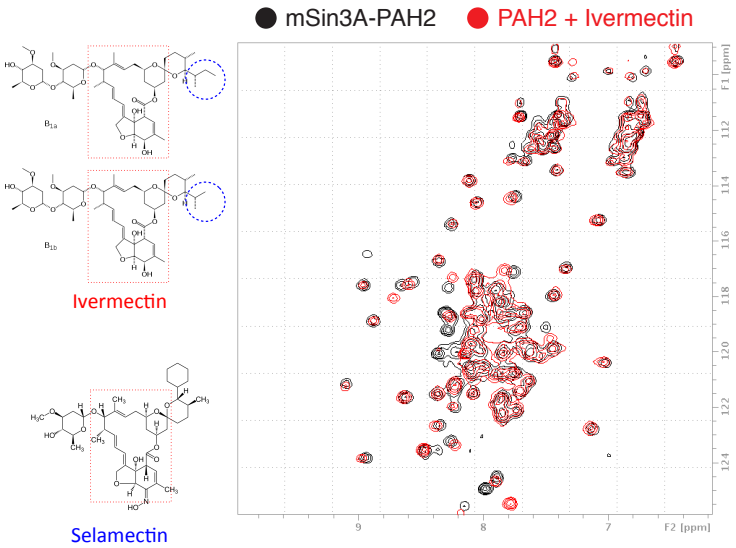

Figure 1. Chemical inhibitors for the mSin3A-PAH2 domain. 2D ^{1}H-^{15}N-HSQC spectra of the ^{15}N-labeled mSin3A-PAH2 domain spectra showing chemical shift perturbations of the protein NMR resonances (black) upon addition of ivermectin (red). Left, chemical structures of ivermectin and selamectin.

indicting direct interactions of the compound with the protein. Given the structural similarity between ivermectin and selamectin, it is likely that selamectin can also interact with the protein, which explains its similar inhibitory effects as ivermectin in our cell-based study (see Aims 1 and 2).

(B) Determination of the structural basis of ligand recognition by mSin3A PAH2 domain - To study the structure-activity relationship (SAR), we have recently determined 3D structures of the mSin3A PAH2 domain in complex with MAD SID peptide, as well as our lead compounds MS6048905 and MS6241743 (see **Figure 2A-C**). New insights into the detailed molecular basis of ligand recognition emerged from these new structures have allowed us to better understand how the PAH2 domain is engaged in interactions with its biological ligand, as well as small molecule inhibitors. Notably, the new structures reveal that the C-terminal α-helix plays an important role in ligand recognition through conformational change upon binding to different ligands. Specifically, the very C-terminal segment of the protein showed an extended α-helical conformation in the absence of a ligand (free state, data not shown), of which side chains of amino acid residues cover the peptide binding site. Unlike the peptide-bound form, in the small molecule-bound forms, the extended C-terminal α-helix retains its conformation, and residues in this segment are engaged in direct interactions with the small molecule compound of MS6048905 or MS6241743. It is such new detailed structural insights that enable us to perform rational ligand design to optimize affinity as well as selectivity of our small molecule lead compounds through exploring additional unique interactions with the target protein through chemical modifications. This is the focus of our research going forward in this project.

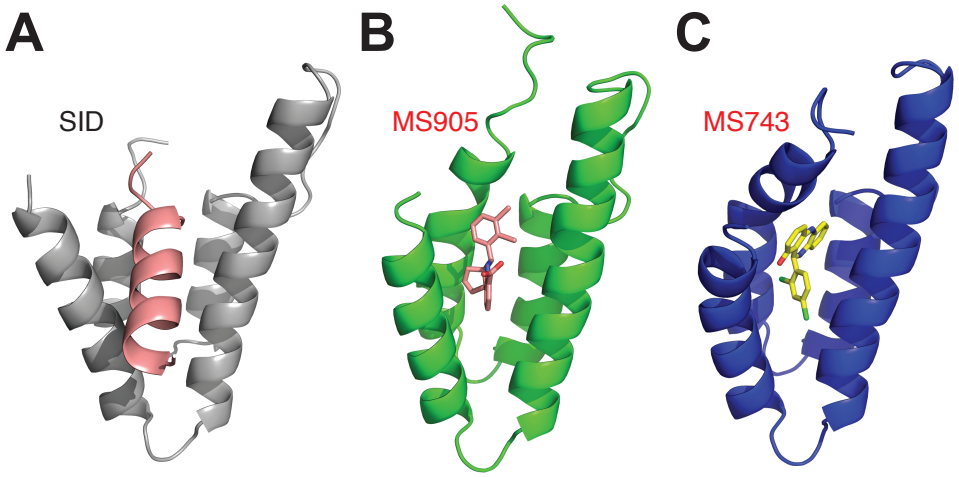

Figure 2. Structure-guided design of small molecule inhibitors for the mSin3A-PAH2 domain. (**A**), **(B)**, **(C)** New 3D solution structures of the mSin3A-PAH2 domain bound to MAD SID peptide, small molecule compound MS905 (MS6048905), or MS743 (MS6241743), respectively, as determined using NMR spectroscopy.

ONGOING WORK FOR 2014-2015:

1. Generating new structure-activity relationship knowledge of MS6048905 and MS6241743 lead series to guide lead optimization.
2. Determining structural/molecular basis of new ligand recognition by the mSin3A-PAH2 domain.

Manuscript in Preparation:

Yeon-Jin Kwon, Boris Leibovitch, Lei Zeng, Mihaly Mezei, Rossitza Christova, Shuai Yang, Rajal Sharma, Edgardo Ariztia, Nidhi Bansal, Ming-Ming Zhou, Arthur Zelent, Eduardo Farias, Samuel Waxman (2014) Selamectin and Ivermectin are small molecule inhibitors that interact with Sin3-PAH2 and exert anti-tumor activity in triple-negative breast cancer. *In preparation.*

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