56th ANNUAL MIDWEST STUDENT BIOMEDICAL RESEARCH FORUM

Saturday, March 8, 2025

ROOM 3042

**10:00 a.m.*	*O-20*	*HOMOLOGOUS RECOMBINATION-RELEVANT PHOSPHOMIMETICS OF REPLICATION PROTEIN A* *Presenter: Valerie Fousek-Schuller*
10:15 a.m.	O-24	STRUCTURAL ELUCIDATION OF THE CATALYTIC SHIFT FROM SUPEROXIDE DISMUTASE TO PEROXIDASE ACTIVITY IN FE-SUBSTITUTED HUMAN MNSOD Presenter: Miles Graham
10:30 a.m.	O-59	GENE LOOP DYNAMICS THROUGH HIPORE-C Presenter: Joynob Akter Puspo
10:45 a.m.	O-61	CONNECTIVITY MAPPING DRIVEN DISCOVERY OF A NOVEL COMBINATION APPROACH FOR GLIOBLASTOMA Presenter: Indumati Ramireddy
11:00 a.m.	O-56	DELETION OF C1GALT1 INDUCES THE METABOLIC REWIRING OF PANCREATIC DUCTAL ADENOCARCINOMA PROGRESSION Presenter: Wyatt Petersen
11:15 a.m.	O-79	IDENTIFYING THE MOLECULAR DETERMINANTS OF METASTATIC ADAPTATION IN PROSTATE CANCER Presenter: Grace Waldron
11:30 a.m.	O-15	TRANSCRIPTIONAL CONTROL OF ERYTHROPOIESIS VIA A KIT-RESPONSE CISTROME Presenter: Venkatasai Rahul Dogiparthi
11:45 a.m.		**LUNCH**

**No longer Presenting*

HOMOLOGOUS RECOMBINATION-RELEVANT PHOSPHOMIMETICS OF REPLICATION PROTEIN A

Valerie J. Fousek-Schuller*, Lucas Struble*, Mona Al-Mugotir, Katelyn Slobodnik, Sneha Pandithar, Savanna Wallin, & Gloria E.O. Borgstahl (UNMC, Omaha, NE, USA) **Equal contributions*

Background, Significance, and Hypothesis

Homologous recombination (HR) repairs DNA double-strand breaks (DSBs), which can be deficient in cancer cells. Heterotrimeric replication protein A (RPA), the main single-stranded DNA-binding protein in humans, is essential for cellular DNA metabolism and damage signalling. It has been known that several N-terminal residues of RPA32 are phosphorylated in the DNA damage response (DDR) and, more recently, that RPA70 and RPA14 are also phosphorylated. RPA is indispensable for HR-based DSB repair, and phosphorylation regulates activity. Both RPA and RAD52 are critical for single-strand annealing. Our studies show that the hyperphosphorylation of RPA regulates the structure and function of the RPA:RAD52 complex. RPA phosphorylation controls "hand-off," or which protein holds the ssDNA. Due to its importance, complexity, and limited understanding, we have continued to study the hyperphosphorylated forms of RPA using phosphomimetics. *We hypothesize that the phosphorylation of RPA is a regulatory mechanism with a structure-function relationship*.

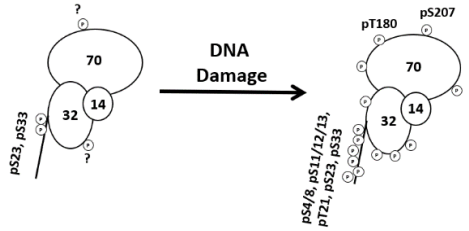

Figure 1. Phosphorylation of RPA before and after DNA damage.

Experimental Design and Results

Our previous studies showed HR-relevant phosphorylation sites on RPA heterotrimers in the G2 and S phases. RPA is always a phosphoprotein, and in control cells, the heterotrimer had three major isoforms having 1, 2, or 4 phosphates, respectively. In DNA-damaged cells, the number of isoforms increased to nine, including up to fourteen phosphorylation sites, as shown in **Fig 1**. Previously, eleven candidate DDR phosphorylation sites on RPA70 and RPA32 were determined and systematically mutated to Glu, creating 6 RPA heterotrimers containing phosphomimetics combinations that were explored. Effects on the activity of these RPA phosphomimetic heterotrimers were evaluated with surface plasmon resonance (SPR) to determine the binding affinity of RPA to ssDNA and RAD52. Significant changes were observed between the mutants when binding ssDNA and RAD52. RPA secondary structures of WT and mutants were explored with circular dichroism (CD), which also showed significant differences between the mutants. Future studies will focus on phosphorylation's regulatory and functional role on the macromolecular complexes that process and repair DSBs.

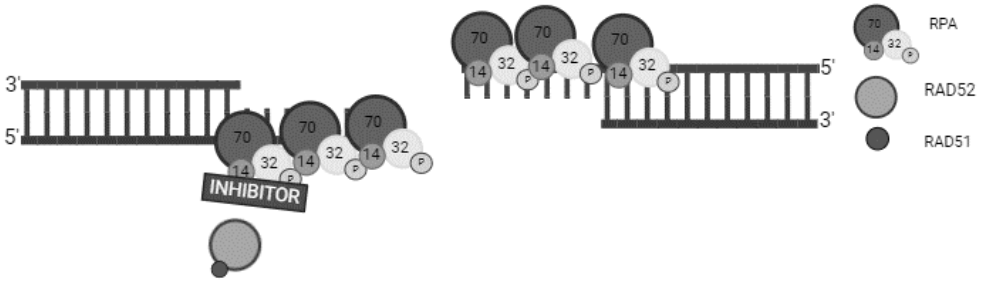

Figure 2. Proposal for inhibiting RPA:RAD52 interaction.

Conclusion

RPA phosphorylation is critical in DNA metabolism, specifically in DNA repair. Understanding how phosphorylation of RPA regulates these various pathways in DNA repair, we can create phosphomimetics for the RPA:RAD52 interaction that could inhibit this interaction involving the alternate HR pathways in BRCA2 complex deficient cells while NOT affecting normal cells, as shown in **Fig 2**.

STRUCTURAL ELUCIDATION OF THE CATALYTIC SHIFT FROM SUPEROXIDE DISMUTASE TO PEROXIDASE ACTIVITY IN FE-SUBSTITUTED HUMAN MNSOD

Miles Graham and Gloria E. O. Borgstahl
University of Nebraska Medical Center, Omaha, NE

Background

Human manganese superoxide dismutase (SOD2) is a metallo-oxidoreductase localized to the mitochondrial matrix. Its canonical function is to scavenge superoxide radicals released from the electron transport chain (ETC) and dismutes them into hydrogen peroxide and molecular oxygen via a proton-coupled electron transfer (PCET) mechanism. This reaction prevents superoxide-mediated oxidative damage to sensitive metabolic enzymes and promotes redox signaling through the release of hydrogen peroxide.

$$Mn^{3+} + O_2^{\cdot-} \leftrightarrow Mn^{2+} + O_2$$
$$Mn^{2+} + O_2^{\cdot-} \leftrightarrow Mn^{3+} + H_2O_2$$

If sufficient Mn is unavailable, such as during Mn deficiency or SOD2 overexpression, Fe can be incorporated into SOD2's active site (FeSOD2). When this happens the antioxidant dismutase activity is entirely abolished and replaced with prooxidant peroxidase activity.

$$Fe^{3+} + H_2O_2 \rightarrow OFe^{4+} + H_2O$$
$$OFe^{4+} + 2e^{\mp}2RH \rightarrow Fe^{3+} + H_2O + 2R^{\cdot}$$

Significance

The downregulation of SOD2 promotes tumorigenesis, while upregulation promotes increased malignancy in established tumors. Fe incorporation is the most likely explanation for this apparent contradiction, with SOD2 overexpression leading to FeSOD2-mediated redox signaling disruption. Furthermore, FeSOD2 highlights large gaps in our knowledge of redox biochemistry; despite the existence of functional Fe-containing superoxide dismutase enzymes in bacteria that are structurally similar to SOD2, FeSOD2's chemistry is radically different for unknown reasons. By elucidating this mechanism, we hope to improve our understanding of redox biology and present a potential therapeutic target for SOD2-enriched malignancies.

Question and Hypothesis

The molecular basis for FeSOD2's mechanistic shift is currently unknown. To elucidate this mechanism, we seek to answer 2 primary questions: why does Fe incorporation change the mechanism and what are the PCETs associated with this altered catalytic cycle? We hypothesize that the incorporation of Fe alters the electronic landscape of SOD2's active site in such a way that the PCETs required to catalyze the superoxide dismutase reaction are not energetically favorable, instead favoring new PCETs that catalyze the peroxidase reaction.

Experimental design

Hydrogenated and perdeuterated FeSOD2 were purified from BL21(DE3) E. coli cultures grown in H2O and D2O, respectively. X-ray crystallography was conducted on hydrogenated FeSOD2 crystals to provide a foundation for neutron crystallographic studies on perdeuterated FeSOD2, which will pinpoint proton positions in the active site. Additionally, X-ray absorption spectroscopy (XAS) will be performed on concentrated hydrogenated FeSOD2 solutions to examine the active site's electronic state. All experiments focus on oxidized, reduced, and substrate-bound FeSOD2 to detail the entire catalytic cycle at the atomic level.

Results

X-ray crystallographic structures of reduced, oxidized, FeSOD2 were collected and showed differences in Fe coordination state, indicative of the expected change in oxidation. The X-ray structure of substrate-bound FeSOD2 shows the presence of a dioxygen species, presumed to be peroxide, bound to the metal center. ICP-MS results confirm the metal present within the active site is Fe and not Mn.

Conclusions

Our X-ray crystallography data shows we can successfully manipulate the redox state of FeSOD2 crystals, and that the substrate is present within the active site. These results are encouraging for our future neutron crystallography and indicate a high likelihood of success. Furthermore, differences in orientation and binding distance observed between the dioxygen ligands of different active sites in the substrate-bound structure may show different points in the catalytic cycle, but this cannot be confirmed until XAS studies are performed.

GENE LOOP DYNAMICS THROUGH HIPORE-C

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Background, Significance, Hypothesis: Chromatin architecture is a dynamic and tightly regulated system essential for transcriptional control. Interaction between transcription start sites (TSS) and transcription termination sites (TTS) can form 'Gene loops' which play a crucial role in coordinating transcription elongation. These loops are established during active transcription driven by the elongation activity of RNA Polymerase II (RNAPII) and stabilized by the cohesin complex. Previous studies have identified gene loops as a feature of chromatin architecture, such as in *Drosophila melanogaster*, but the dynamics of formation, regulation, and functional implications in humans remain poorly understood.

Advancements in mapping technology, such as Hi-C have revealed chromatin interactions on a genome-wide scale but lack the resolution and specificity required to capture fine-scale, multiway, or allele-specific chromatin interactions. HiPore-C, a novel long-read sequencing platform, addresses these limitations by providing a high-resolution multiway contacts view of chromatin structure. This technology offers an opportunity to study the dynamic interplay between transcription and chromatin organization. The ability to dynamically map gene loop formation and chromatin reorganization is essential for understanding how transcription influences chromatin compartments and borders. This knowledge could provide critical insights into transcriptional dysregulation in diseases, such as cancer, and inform therapeutic strategies targeting chromatin modifiers. However, it is unclear if gene loops are restricted to TSS-TTS connections or involve complex multi-loop interactions along the gene body. Furthermore, the link between RNAPII progression, compartment transitions, and loop formation is not well understood

We hypothesize that RNAPII elongation drives gene loop formation, dynamically reshaping chromatin from inactive to active compartments and influencing transcriptional regulation. These loops may extend beyond simple TSS-TTS interactions, forming multiple loops within the gene body and creating a crumpled chromatin structure during active transcription.

Experimental Design: To test our hypothesis, we performed a series of time-course experiments. HCT116 cells were serum-starved for 48 hours to halt transcription and to synchronize transcriptional activation. To capture the dynamic stages of transcription elongation we reintroduce serum at intervals of 0, 25, 50, and 125 minutes. RNAPII Ser2P ChIP-seq was used to track RNAPII progression along the gene body during elongation. To provide preliminary evidence of dynamic loop formation, Hi-C experiments were performed at the same time points to map pairwise chromatin interactions.

To resolve multiway chromatin interactions, we performed HiPore-C targeting the same time points on the Nanopore sequencing platform where we incorporated size selection for ligated DNA fragments over 3 kb to enrich longer fragments and thus maximized multiway contact detection. This approach enabled to distinguish single-loop interactions from potential multi-loop crumpling within the gene body and facilitated detailed investigation of multiway chromatin interactions, linking RNAPII progression to gene loop formation and chromatin compartment transitions.

Data and Results: Our RNAPII Ser2P ChIP-seq showed the progression of RNAPII along the gene body during transcription elongation, with distinct peaks at the TSS, gene body, and TTS at different time points. This confirmed the temporal dynamics of RNAPII activity during transcription. Hi-C data showed consistent interactions between TSS and TTS across all time points (0, 25, 50, and 125 minutes) which provide evidence of stable pairwise chromatin contacts. Our compartmental analysis indicated that RNAPII elongation caused dynamic shifts, with TSS regions predominantly in the active (A) compartment and TTS regions transitioning between inactive (B) and active compartments over time.

The HiPore-C data from this study provided high-resolution insights into chromatin interactions. HiPore-C generated 30 million pairwise contacts from 1 million sequenced read, highlighting its efficiency compared to Hi-C, which requires nearly double the reads for similar data. This also indicates multiway chromatin interactions, meaning that single genomic regions often engaged with multiple sites. This suggests the presence of complex looping structures or crumpling within the gene body that are inaccessible to traditional pairwise analysis.

Conclusion: The results support the hypothesis that RNAPII progression forms gene loops and reshapes chromatin borders. Using HiPore-C, this study captured high-resolution multiway chromatin interactions, revealing complex looping patterns that provide insights into transcriptional elongation and chromatin organization. However, additional replicates and deeper sequencing are needed to strengthen these findings and fully explore the complexities of gene loop dynamics and their role in transcriptional regulation.

CONNECTIVITY MAPPING DRIVEN DISCOVERY OF A NOVEL COMBINATION APPROACH FOR GLIOBLASTOMA
op_graduate_ramireddy_indumati_abstract.pdf

***Ramireddy Indumati*[1]*, Pranita Atri*[1]*, Zahraa Wajih alsafwani*[1]*, Maneesh Jain*[1,2]*, Raghupathy vengoji*[1.]*, Surinder K. Batra*[1,2,3]*, Nicole Shonka*[1,2,4]

[1]Department of Biochemistry and Molecular Biology,[2] Fred and Pamela Buffet Cancer Center, [3]Eppley Institute for research in Cancer and Allied Diseases,[4]Department of Internal Medicine, Division of Oncology and Haematology, University of Nebraska medical Center, Omaha

Background: Glioblastoma (GBM) is an aggressive grade 4 primary malignant tumor of the central nervous system, with a poor mean overall survival (mOS) of 12-18 months post-diagnosis. Of the 14,000 malignant glioma cases diagnosed annually in the U.S., 60-70% are GBM. The current standard of care for GBM includes maximal safe surgical resection, radiotherapy, with concomitant and adjuvant chemotherapy using temozolomide (TMZ), along with tumor-treating fields. However, despite these treatments, tumors typically recur within 6.9 months due to cellular heterogeneity and multiple oncogenic pathways that drive tumor resistance. The activation of the Janus kinase 2 /Signal transducer and activator of transcription factor 3 (JAK2/STAT3) signaling pathway is a central focus of tumorigenesis in GBM. In addition to conferring many tumor-promoting features, STAT3 signalling also regulates the expression of MGMT (O-6-methyl guanine-DNA-methyltransferase), a key player in dictating therapy selection and outcome by mitigating TMZ cytotoxicity, thus contributing to resistance. Therefore, tight control of JAK2/STAT3 activity is crucial to preventing malignant transformation and overcoming resistance.

Significance: Our group has identified a potential FDA-approved, blood-brain permeable drug, tg101348/fedratinib, a JAK2 inhibitor, through Connectivity Mapping (CMap). Fedratinib effectively decreases MGMT expression among other FDA-approved JAK inhibitors. Furthermore, evaluating the efficacy of FDA-approved drugs, such as fedratinib, provides potential strategies to counteract the resistance encountered in GBM.

Hypothesis: We hypothesize that inhibiting the JAK2/STAT3 pathway, a major oncogenic signalling pathway implicated in GBM, may decrease therapy resistance, and improve patient survival rates.

Experimental design: Datasets (GSE61335, GSE35493, GSE50161, & GSE13276) from the NCBI Gene Expression Omnibus (GEO) repository were used to analyse both normal (total N = 38) and tumor samples (total T = 99). The differentially expressed genes were then subjected to CMap, a bioinformatic tool, to identify drugs negatively connected to the GBM signature (-80% efficacy) that could reverse the tumor profile. Fedratinib was identified as a top candidate. The efficacy of fedratinib, both alone and in combination with TMZ, was evaluated in vitro using human GBM cell lines (U251, U118, LN18), a mouse syngeneic line EPG (EGFRvIII, p16-/-, GFAP Cre), and patient-derived cultures (CK9495). Experiments were repeated at least three times, and results are presented as mean ± SD. Statistical analysis was performed using SAS 9.4, with P-values < 0.05 considered statistically significant.

Results: Fedratinib decreased cell viability in a concentration-dependent manner. Furthermore, combining fedratinib with TMZ produced a synergistic effect, significantly enhancing therapeutic efficacy compared to fedratinib alone. Treatment of both human and mouse syngeneic cell lines with fedratinib + TMZ led to a marked inhibition of STAT3 (Tyr705) activation and significantly increased apoptosis. Additionally, this combination therapy effectively decreased the expression of cancer stemness markers, which are critical for tumor initiation and resistance. Moreover, the combination showed superior potential to inhibit the activation of other STAT isoforms, such as STAT3 (Ser727) and STAT1 (Tyr701), which contribute to GBM tumor progression. Importantly, the combination also resulted in a significant reduction in MGMT expression. Notably, the synergistic drug duo was non-toxic to non-transformed cells, including human and mouse astrocytes and endothelial cells**.**

Conclusions: In conclusion, preliminary data suggest that targeting the JAK2/STAT3 pathway with fedratinib in combination with TMZ may enhance treatment efficacy and overcome resistance in GBM. These findings warrant further investigation to assess their potential for improving patient outcomes.

DELETION OF C1GALT1 INDUCES THE METABOLIC REWIRING OF PANCREATIC DUCTAL ADENOCARCINOMA PROGRESSION

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Background and Significance of the Problem

Pancreatic cancer (PC) is one of the deadliest malignancies, ranking among the leading causes of cancer-related deaths worldwide due to its aggressive progression and late-stage diagnosis. Despite advances in treatment, the prognosis remains dismal, with limited therapeutic options and high rates of metastasis. Among the molecular alterations driving this disease is aberrant O-glycosylation, a process characterized by the expression of truncated carbohydrate antigens such as Tn and sialyl-Tn (STn). These glycan modifications are widely associated with poor prognosis and enhanced metastatic potential in PC.

A key enzyme in O-glycosylation, core 1 β1,3-galactosyltransferase (C1GALT1), catalyzes the biosynthesis of core 1 mucin-type O-glycans, but its inactivity in PC leads to the accumulation of truncated glycans. Analysis of poorly differentiated human PC tissues revealed a significant loss of C1GALT1 expression. CRISPR/Cas9-mediated knockout (KO) of C1GALT1 in PC cell lines also results in increased Tn and STn glycan expression, enhanced tumorigenic potential, and increased metastatic capacity. Using genetically engineered mouse models (*C1galt1* KO with $Kras^{G12D}$/$Trp53^{R172H}$/+ mutations), we observed early-onset and widespread metastasis, mirroring the heightened aggressiveness associated with C1GALT1 loss in pancreatic cancer. However, the mechanisms by which the loss of C1GALT1 drives progression in pancreatic ductal adenocarcinoma remain underexplored, offering an opportunity to identify novel therapeutic targets in this highly aggressive cancer type.

Hypothesis

We hypothesize that the loss of C1GALT1 in Pancreatic Ductal Adenocarcinoma facilitates tumor progression through the induction of metabolic rewiring.

Experimental Design

To investigate this hypothesis, we conducted transcriptomic profiling through RNA sequencing and pathway enrichment analysis to identify metabolic pathways altered by C1GALT1 knockout (KO) in pancreatic cancer cells. These findings were validated using qPCR, ddPCR, western blotting, and immunohistochemistry (IHC) to confirm the expression changes and pathway alterations. Additionally, functional assays, including wound healing studies, were performed to evaluate the impact of C1GALT1 loss on cellular motility and metabolic alterations.

Results/Data

Transcriptomic profiling through RNA sequencing and pathway enrichment analysis identified significant alterations in metabolic pathways following C1GALT1 knockout (KO) in pancreatic cancer cells, including a notable upregulation of the cholesterol metabolism gene SQLE. These findings were validated using qPCR, ddPCR, western blotting, and immunohistochemistry (IHC) to confirm changes in gene expression and pathway activation. Additionally, functional assays, including wound healing studies, demonstrated enhanced cellular motility in C1GALT1 KO cells.

Conclusions

This study underscores the critical role of C1GALT1 loss in driving pancreatic cancer progression through both metabolic reprogramming and functional adaptations. By linking aberrant glycosylation to altered cholesterol metabolism and enhanced cellular motility, our findings shed light on the molecular mechanisms underlying the aggressive behavior of C1GALT1-deficient pancreatic tumors. These results provide novel insights into the interplay between glycosylation and metabolism in cancer and highlight potential therapeutic vulnerabilities. Targeting the metabolic pathways associated with truncated glycans may offer a promising approach to disrupt the aggressive phenotype of pancreatic cancer and improve patient outcomes.

IDENTIFYING THE MOLECULAR DETERMINANTS OF METASTATIC ADAPTATION IN PROSTATE CANCER

Grace Waldron, Chrystal Nathan, Diane Costanzo-Garvey, Heather Jensen-Smith, Grinu Mathew (UNMC Omaha, NE)

Background: Prostate cancer (PC) is the number one diagnosed cancer in men in the US and the second most common cancer among men worldwide. In turn, PC leads to a significant public health challenge. It was estimated that approximately 299,010 men in the US will be diagnosed with PC in 2024. Understanding metastasis is crucial due to its impact on disease morbidity. The sites of PC metastasis, such as the bone and visceral organs play key roles in disease morbidity. While bone metastasis is common, visceral metastases is associated with poor survival. The microenvironmental suitability for cancer cells in both bone and visceral organs strengthens their impact on disease progression and treatment outcomes. Further, the exploration of metastatic adaptations involves immune evasion mechanisms, interactions within the tumor microenvironment, and the ability of cancer cells to enter dormancy. Understanding the complexities of PC metastasis, including the molecular mechanisms and adaptation factors, is pivotal for developing effective therapies.

Significance of Problem: Published literature and genetic approaches have uncovered that aggressive metastatic PC display genetic loss of the tumor suppressors *PTEN* and *TP53*. To identify the molecular signature of metastatic *PTEN/TP53*^{NULL} cells we performed rigorous multiOmic/ and biochemical analysis of cells derived from tumors at various stages of disease evolution (*Pten/Trp53*^{NULL} mouse model; RapidCaP). This analysis revealed that loss of receptor tyrosine kinase Axl is tightly correlated with metastatic spread to bone and visceral organs. AXL is a member of the **T**yro3**A**xl**M**ertk family of kinases and has shown to be necessary for inducing dormancy like state in PC cells *in vivo*. Integrating CRISPR-Cas9 technology with robust immunocompetent syngeneic models, our lab has successfully demonstrated that *Axl* knockout promotes a metastatic phenotype in *Pten/Trp53*^{NULL} cells. However, we lack the mechanistic insight on how of *Axl* knockout cells adapt and grow within the metastatic site.

Hypothesis: We hypothesize that the loss of *Axl* in *Pten/Trp53*^{NULL} cells promotes a metastatic phenotype and plays an essential role in metastatic adaptation. We propose that understanding the biological dependencies of *Pten/Trp53/Axl*^{NULL} cells is crucial for therapeutic targeting of metastatic PC.

Experimental Design: To explore this hypothesis we generated a PC metastasis model with *Axl* knockout (KO; CRISPR) in *Pten/Trp53*^{NULL} RapidCaP derived cell lines. To determine if the tumor cells are present and proliferating within the lung tissue, a DHB-mVenus cell cycle sensor was added to the cell lines. The addition of this fluorescent reporter not only allows for the identification of injected *Axl* WT or *Axl* KO cells but can also be used for *in-vitro* and *in-vivo* cell cycle analysis experiments. To optimize the model further, both the *Axl* WT and *Axl* KO cell lines were transfected with the firefly-luciferase gene for tracking disease progression in vivo (bioluminescence imaging). Next, we generated a lung metastasis model (syngeneic) via tail vein injection of *Axl* KO (DHB-mVenus/Luciferase) or control *Axl* WT (DHB-mVenus/Luciferase) cells. These *in-vivo* trials enabled the exploration of biological differences between *Axl* KO and WT cell lines using transcriptomics, immunohistochemistry and multiphoton-photon microscopy-based collagen imaging.

Results/Data: From the current data, we have been able to demonstrate that *Pten/Trp53/Axl*^{NULL} PC cells display faster growth of overt lesions and increased metastatic burden when compared to *Axl* WT cells; based on immunohistochemical presentation within mouse lung tissue. On the contrary, the *Axl* WT cells formed fewer and smaller lesions and prolonged the overall survival of tumor bearing mice. Transcriptomic analysis of *Axl* KO vs WT cells revealed an enrichment of inflammatory response pathway, increased MTORC1, KRAS signaling and upregulation of protein secretion pathways.

Conclusion: Overall, we have been able to establish a metastatic disease model and began characterizing the biological differences of the model. We have observed that *Axl* KO cells developed overt metastasis within three weeks whereas the *Axl* WT cells remain repressed, exhibiting disseminated tumor cells as detected by GFP staining. Further studies need to be conducted to understand the role of the microenvironment and immune systems role in the maintenance of metastatic disease within this model.

TRANSCRIPTIONAL CONTROL OF ERYTHROPOIESIS VIA A KIT-RESPONSE CISTROME

Venkatasai Rahul Dogiparthi, Linda Chee, M. Jordan Rowley, and Kyle J. Hewitt

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ABSTRACT

Cis-regulatory elements (*cis*-elements) interact with transcription factors to control when and how genes are expressed in response to specific extracellular stimuli. Chromatin signatures at *cis*-elements can be used to predict their activities and mechanisms of transcriptional control. However, accurate cell type-specific prediction of functional *cis*-elements remains a significant computational challenge due to the lack of ideal datasets focused on molecular features and locus-specific sequence. Moreover, static predictions of activity do not evaluate the potential for *cis*-element responses to extracellular growth factor stimulation. Despite these limitations, clinically-relevant *cis*-elements regulating hematopoietic processes have been identified using these approaches, leading to new treatments for sickle cell disease and screening for susceptibility to myelodysplastic syndromes.

To improve predictions of *cis*-element activity downstream of specific cell signaling pathways, we developed a multi-step approach to annotate, rank, and functionally-validate *cis*-elements. We chose to evaluate predictive features of *cis*-element activity at nearby or interacting regions with Kit-regulated genes (termed “Kit response element”, KRE), since this signal is essential for hematopoietic and erythroid progenitor cell (EPC) survival, proliferation, and lineage commitment. **We hypothesize that Kit-response cis-elements (KREs) are required for transcription and EPC activity.** RNA-seq data in acutely Kit-stimulated cells was filtered by Kit-Activated transcripts and annotated for potential nearby or interacting *cis*-elements. We then trained a random forest machine learning model on multifactor prioritization criteria (transcription factor occupancy, histone modifications, and predicted activity) compared to Kit-insensitive control regions. The overall accuracy of this model at predicting Kit-response loci was 89%. Among 750 possible KREs, 45 Kit-predictive features were identified and used to rank KREs based on confidence scores. The top Kit-predictive features included epigenetic regulators (ARID1B, SMARCA4, TBL1XR1, TCF12), Inflammatory Response (JUN, JUND), and blood cell maintenance (NCOR1, CBFA2T2, CBFA2T3). We generated ATAC-seq data post Kit-activation and performed motif footprint analysis. This revealed increased enrichment of Kit-activated inflammatory response transcription factors’ footprints including Early Growth Response-1 (EGR1), known to regulate cell proliferation and differentiation and 37-fold upregulated in response to Kit pathway activation.

To test whether EGR1 transcriptional upregulation is required in a Kit-dependent genetic network, we used a CRISPRi (dCas9-kRAB) model to prevent Kit from upregulating *EGR1* (8-fold, $p<0.0001$). HUDEP-2 cell expansion rate decreased 2.5-fold in sg*EGR1* vs controls. We identified 550 EGR1-sensitive KREs using our previously implemented *in silico* approach. EGR1-sensitive predictive features included occupancy of transcription factors (GATA1, GATA2, LDB1) and epigenetic regulators (SMARCB1, KDM6A, CHAMP1, EHMT2). 47 EGR1-Sensitive KREs showed enrichment for key EPC transcription factors’ (KLFs, SPs, CTCF) footprints at EGR1-sensitive KREs in our ATAC-seq data. Given the EGR1 dependency for select Kit pathway transcriptional activity, we are testing locus-specific mechanisms in EGR1-dependent and -independent transcription for EPC activity.

Impaired Kit signaling resulting from Kit receptor mutations causes hematologic diseases including leukemia. While many cellular outcomes of Kit signaling are established, chromatin targets of this pathway are poorly understood. With tumors developing resistance to inhibitors, it is critical to understand transcriptional targets at KREs that are required for Kit signaling activity. Many of our predicted KRE target genes have poorly described or unknown roles in erythropoiesis. Annotating KRE function will improve future predictive models and identify targets for drug development to treat Kit-related malignancies.