Introduction: Ovarian cancer is a devastating disease in women and the current strategies for therapeutic management are not very successful. Our data demonstrate that the changes in the matured form of proteins like Insulin Like Growth Factor 1 Receptor (IGF1R) and Mesothelin compare to the precursor form express high in cancer tissues and cell lines. Furin is a cellular endoprotease, which helps the maturation of several important precursor form of protein substrates through its proteolytic actions. However, the role of Furin as pro-cancer proteolytic enzyme is not well studied. In this study, we identified that Furin-mediated maturation of IGF1R protein is critical for the progression and metastasis of ovarian cancer through STAT3 signaling.

Objective: Furin is an understudied proteolytic enzyme in ovarian and other gynecological malignancies. In this study, we will elucidate the mechanism of action how Furin promotes ovarian cancer progression and metastasis as well as determine the effects of inhibiting Furin to disrupt the progression of ovarian cancer.

Methods: Expression of Furin and its target genes were detected in cancer cell lines and cancer tissues by western blotting, immunohistochemistry and immunofluorescence staining. To determine the role of Furin on tumor cell migration, we used target-specific siRNAs to knockdown Furin in ovarian cancer cell lines and then performed cell migration assay, matrigel invasion assay. Cancer cells were grown in extracellular matrix for 3D culture. To determine the role of Furin in preclinical models, we have knocked down Furin in ovarian cancer cells and functions of ovarian cancer orthotopic implantation animal model was used, followed by biosensor analysis.

Results: In the high-grade ovarian cancer patients, Furin is highly expressed in tumor omentum compared with normal omentum. Meanwhile, Insulin Like Growth Factor 1 Receptor (IGF1R) expression was also upregulated in the patient tumor omentum. When Furin was silenced in different ovarian cancer cell lines, cell migration, cell invasion, spheroids size and numbers were significantly reduced. It is interesting that we also found that Furin knockout downregulated the level of IGF1R and p-stat3(Tyr705). In vivo animal data showed that when Furin was knocked down, tumor weight, tumor nodules and tumor ascites were significantly reduced. We also checked Furin, IGF1R and p-stat3 expressions in animal tissues using immunohistochemistry. All these protein expressions were suppressed in Furin knockdown group.

Conclusions: Our findings yielded additional insights into newly proposed roles for Furin in ovarian cancer progression and metastasis through the maturation of IGF1R and subsequent activation of STAT3 in cancer cells. Our study confirmed that Furin can be a new therapeutic target for ovarian cancer. Our data suggests that inhibiting the levels of Furin could modulate the tumor microenvironment and inhibit the progression and metastasis of ovarian cancer. We are expecting that our studies will improve the understanding on the role of Furin on the maturation of key precursor proteins and their contributions on ovarian cancer metastasis.

Significance: Our study suggested that Furin can be a new therapeutic target for ovarian cancer. We are expecting that our studies will have an impact on other cancers with a high level of Furin expression.

Funding source: Our lab is supported by the grants from The Ovarian Cancer Research Fund Alliance, research funds from Women’s Health Research Program in the Department of Obstetrics and Gynecology (DB(GB)N), and start-up funds from the Department of Obstetrics and Gynecology at the Medical College of Wisconsin.

Objective: Decipher the role of SNRK in promoting ovarian cancer metastasis.

Methods: Ovarian cancer cell lines A2780, SKOV3ipl, CaOV3, Ov90 were used for the study. Stable cell lines were generated with SNRK knock down to study the effect of SNRK. Immunohistochemistry was used to detect the expression of SNRK in primary ovarian tumors, normal omentum and omental metastatic samples. The tumorigenic potential of SNRK was assessed by migration and anchorage-independent proliferation assays. SNRK expression was studied by immunofluorescence imaging and western blotting. Mitochondrial membrane potential was studied by JC-1 staining and the ultrastructure was determined by transmission electron microscopy. Extracellular flux analyzer was used to study the bioenergetic profile and the status of fatty acid oxidation in the cells. Protein association studies were done by co-immunoprecipitation.

Results: Our data shows that SNRK is highly expressed near the tumor-invasive fronts but could not be detected in the advanced metastatic tissue sections. Expression analysis in a panel of cell lines revealed that SNRK shows mediate nuclear localization. SNRK was found to be important for cellular proliferation but was noted to have an inhibitory effect on the migratory potential. Extracellular flux analysis revealed that the proliferation profile was associated with a change in the bioenergetic state of the cells with SNRK knockdown. SNRK seemed to be crucial and necessary for mitochondrial respiration as compared to cellular glycolysis. Studying the mitochondrial physiology revealed that SNRK uncouples mitochondrial respiration thereby dereting ATP production. Mitochondrial ultrastructure analysis revealed that SNRK is essential to maintain mitochondria in a fused state to assist the process of fatty acid oxidation. Knocking down SNRK led to the appearance of bigger lipid droplets in the vicinity of truncated mitochondria. To study the role of extracellular fatty acids on the expression of SNRK, we treated ovarian cancer cells with palmitic acid and found that palmitic acid led to an increase in SNRK expression in a dose dependent manner. SNRK was found to associate with Salt-inducible Kinase 2 (Sk2), which is reported to be necessary for the initial stages of ovarian cancer metastasis.

Conclusions: Ovarian cancer cells display heterogeneity in their energetic behavior which accounts for their dependence on SNRK. Cells which are highly energetic (relly both on glycolysis and mitochondrial respiration) are more vulnerable to SNRK-mediated changes as compared to glycolytic cell lines. This implies that SNRK is a crucial regulator of mitochondrial respiration. SNRK uncouples mitochondrial respiration thereby restricting the ATP supply and propelling the cells to depend on alternate sources for energy production.

Significance: Our data puts forward a novel role of SNRK in the initial stages of ovarian cancer metastasis. Inhibition of SNRK might help in developing new therapeutic regimens to restrict ovarian cancer progression and metastasis.

Funding source: Wisconsin Ovarian Cancer Alliance, Cancer Center Pilot Grant, and Cancer Center Support Grant (NCI CA212097)
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Basic
FXR1‐mediated Oncogenic Adaptations via Post Transcriptional Regulation of c‐MYC
Ovarian Cancer Research Fund Alliance (OCRA), DoD Breast Cancer Research Program (W81XWH-18-1-0024), the Women’s Health Research Program (NHBP) at MCW, American Cancer Society’s IRC, and research funds from MCW Cancer Center.

Introduction: Breast and ovarian cancers are the most lethal malignancies in women. Our data shows that Copy Number Variations of RNA binding proteins (RBPs) changes the post‐transcriptional events contributes towards tumor initiation and metastasis. However, the functional role of RBPs has not been explored carefully in cancer. We identified that an RNA binding protein FXR1 is highly amplified in breast and ovarian cancers and this genomic amplification upregulates the expression of FXR1. In this study we characterized the key targets of FXR1 and its mechanism of action in breast and ovarian cancers. Our analysis and assays identified that c‐MYC is the key target of FXR1 and FXR1 upregulates c‐MYC levels by altering post‐transcriptional and translational mechanisms. We further demonstrated that the upregulation of c‐MYC through FXR1 dysregulates cell cycle, provides metabolic and proliferative advantages, and hijack pro‐apoptotic mechanisms in cancer cells.

Objective: This study aims to evaluate the mechanism, how FXR1 upregulates c‐MYC and other oncogenes for the growth and metastasis of cancer cells.

Methods: FXR1 expression in human breast and ovarian cancer cells and tissues were determined by Western blotting, immunohistochemistry and qPCR. We have either knocked down or overexpressed FXR1 in breast and ovarian cancer cells and performed MTT proliferation assay, colony formation assay, matrigel invasion assay, cell cycle analysis, and real‐time quantitative PCR (qPCR) array. We used this medium through qPCR arrays to determine the expression of cell cycle genes, metabolism genes and the expression of tumour suppressor genes and oncogenes differentially expressed upon the alterations of FXR1. We also performed seahorse biosay to measure to the oxygen consumption and extracellular acidification rate upon FXR1 altered cancer cells. To identify the proteins which interact with FXR1, we performed co‐immunoprecipitation (CoIP) analyses following by immunoblotting. We also performed RNA immunoprecipitation (RIP) to show the interaction between FXR1 with c‐MYC mRNA.

Results: Our analysis identified that FXR1 is highly amplified in several types of human malignancies, particularly in lung, ovarian, cervical and breast cancers. We show that FXR1 is overexpressed in a panel of cancer cell lines and tissues, and the high expression of FXR1 promotes the growth, migration and invasion of cancer cells. In contrast, silencing of FXR1, reduced the proliferation, chlonogenic potential, invasion, and wound healing abilities of cancer cells. We also found that the loss of FXR1 modulated the levels of apoptosis‐related proteins, as well as promoted G‐1 phase arrest. FXR1 silencing also reduced the glucose consumption, lactate secretion, mitochondrial membrane potential and the generation of reactive oxygen species (ROS). Importantly, we found that FXR1 expression associate with poor outcome of breast, ovarian and lung cancer patients.

Our data revealed that all the oncogenic effects mediated by FXR1 are orchestrated through c‐MYC. We further demonstrated that the upregulation of c‐MYC through FXR1 dysregulates cell cycle, provides metabolic and proliferative advantages, and hijack pro‐apoptotic mechanisms in cancer cells. The oncogenic effects mediated by FXR1 are orchestrated through c‐MYC.

Conclusions: Our results demonstrate that the RNA Binding Protein FXR1 is an unexpected driver of breast and ovarian cancers. Our study characterizes the previously unknown roles of FXR1 on the progression and metastasis of cancer cells. Herein, we identified that the oncogenic effects of FXR1 are mediated through c‐MYC oncogene. Importantly, we identified that FXR1 facilitates the recruitment of translation initiation factor complex to the translation initiation site of c‐MYC mRNA and enhances the translation of c‐MYC protein.

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Basic
Astrocyte secreted factors influence medulloblastoma tumor cell phenotypes
The MCW Department of Pediatrics and the Children’s Research Institute.

Introduction: Medulloblastoma (MB) is a pediatric brain tumor that forms in the cerebellum or brainstem of the brain. Various subtypes of the tumor are known to be highly metastatic and have poor outcomes. Our previous work has focused on identifying potential drivers of MB metastasis and we hypothesized that astrocytes, the cellular component of the proliferative zone, play a role in the aggressive behaviour of MB cells. In this study, we aimed to investigate if astrocytes secrete factors that influence MB cell adhesion and invasion.

Methods: To determine the effects of astrocyte secreted factors on MB cells, we utilized media conditioned from the culturing of neonatal rat astrocytes. We analyzed astrocyte conditioned media (ACM) for adhesion and invasive properties on Daoy and UW228 (human MB cell lines) cells. In addition, gene expression was analyzed on Daoy cells cultured in ACM which led to further investigation of the adhesive properties of cells cultured in ACM compared to control media, through cell‐based assays and flow cytometry assessment of cell surface adhesion proteins. A neurosphere formation assay was also performed to assess stem‐like characteristics of the MB cells.

Results: Daoy cell and UW228 cell lines showed increased adhesion and invasion in ACM in the presence of ACM. Gene expression analysis comparing normally cultured and ACM cultured Daoy cells revealed several adhesion related genes to be upregulated, including L1CAM, NCAM2, and NAFASC. TGFβ‐2 is known to be a regulator of these adhesion proteins and has also been shown to be secreted by astrocytes. When Daoy cells were treated with TGFβ‐2, however, both the adhesion proteins and adhesion remained unchanged. Interestingly, we did find that both TGFβ‐2 and ACM upregulated apotosis and CD133 in Daoy cells. In addition to being associated with adhesion and proliferation formation, CD133 is a known marker for MB stem‐like cells.

Conclusions: The results of this study indicate that astrocyte signaling mechanisms facilitated by the secretion of factors can be co‐opted by MB cells to promote expression and morphology changes that may lead to more aggressive tumors, such as increased invasion and proliferation. Additionally, the role of CD133 in cell survival, or resisting apoptosis, is being evaluated. Although no difference in displacement of the Daoy cells was seen in the 24‐hour time‐lapse imaging experiment, the increase in protrusion formation indicates that the cells were primed to migrate. Significance: Since astrocytes are such a large component of the MB microenvironment, defining their contribution to pediatric tumor development will facilitate future targeting strategies. In this study, ACM increases invasion, adhesion, and enriches CD133 expressing MB cells. CD133 is often localized in cellular protrusions, and therefore this association and its meaning is currently being studied in the lab. In addition, the increase in CD133 in neurosphere formation indicates that ACM may be enriching cells that have increased stem‐like and self‐renewal potential.

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Basic
Acetylation activates an alternative function of SOD2 in breast cancer
NIH/NCI ROICA126882

Mitochondrial superoxide dismutase (SOD2) displays a dichotomous role in cancer, being a suppressor of tumor initiation while stimulating cancer progression later in the established disease. The mechanistic basis of this switch remains unknown. Our results indicate that an increase in SOD2 expression beyond a defined threshold leads to its accumulation in an acetylated state and the activation of redox stress responses including, as reported here, the activation of hypoxic signaling. Specifically, we found that increased expression of SOD2 leads to SOD2 stabilization and hypoxia‐induced factor 2α (HIF2α) in a HD2‐dependent manner. Consequently, the SOD2/ HIF2α promotes core stemness gene (i.e. CXCR4 and SOX2) expression increasing the cancer stem cell (CSC) subpopulation, tumorigenicity and invasiveness of breast cancer cells. Based on these findings we propose that as tumors evolve to advanced stages a mitochondrial pathway to stemness reprogramming dependent on SOD2/ROS and HIF2α is activated. It is also proposed that SOD2/HIF2α+ signature may identify cells in breast cancer with tumor initiating capacity and high metastatic potential.
Introduction: Acute myeloid leukemia (AML) is an aggressive malignancy with a 5-year survival of less than 50%. Within AML, even including distinct subtypes, there is significant genetic heterogeneity. Different chromosomal translocations involving the core-binding factor complex are well-known “drivers” of leukemia development and have very similar clinical and prognostic implications. The core binding factor complex is essential for normal hematopoiesis and is composed of two subunits, AML1 (also known as RUNX1) and CBFB. Both AML1 and CBFB are involved in distinct chromosomal translocations in AML, t(8;21) and inv(16) respectively, which generate the fusion oncoproteins AML1-ETO or CBFB-MYH11. Surprisingly, these additional mutations are not found in animal models, suggesting additional mutations in one of four members of the core-binding complex (RAG2, SMC3, STAG2, and SMCA1) are commonly found in patients with AML, and frequently (up to 25%) co-occur with AML1-ETO, but never with CBFB-MYH11. This suggests a strong selective pressure for the presence or absence of cohesion mutations depending upon the driver oncogene. Specifically, we hypothesize that cohesion mutations synergize with AML1-ETO during leukemic transformation, whereas CBFB-MYH11 and cohesion display a synthetic lethal genetic interaction.

Objective: In this study, we set out to determine the relationship between cohesion haplosufficiency and core-binding factor oncogenes in AML development. By learning more about the mechanism by which these driver oncogenes form distinct genetic interactions with cohesion, we may be able to develop precision medicine approaches.

Methods: To explore the relationship between cohesion haplosufficiency and core-binding factor complex mutations we have engineered murine-derived bone marrow cells that express the oncogenic fusion proteins that are either Smc3+/− or Smc3−/−. We have studied the phenotype of these cells in vitro and with next generation sequencing technologies. To gain a better mechanistic understanding we have performed ATAC-seq analyzing the difference on the accessibility of known transcription factor binding sites in AML1-ETO;Smc3+/− compared to AML1-ETO;Smc3−/−. Additionally, we have performed RNA-seq to identify transcriptional differences due to cohesion haplosufficiency and AML1-ETO expression. Results: Our preliminary in vitro studies indicate that the loss of cohesion augments in vitro self-renewal of AML1-ETO and promotes an immature, blast-like morphology. Our ATAC-seq analysis has uncovered several motifs implicated in myeloid development (RUNX1, GATA2, ERG, PU.1), nuclear architecture (CTCF, CTCFL), and cell proliferation (AP-1, FLI1, JUN) as enriched in the Smc3+/− background. RNA-seq revealed deregulation of genes involved in myeloid cell differentiation, changes corresponding to Myc upregulation, and upregulation of the Rb and p53 oncogenic gene signatures in AML1-ETO;Smc3−/− compared to Smc3+/−. Interestingly, we did not identify significant transcriptional differences that correspond to the factors that bind the more accessible motifs.

Conclusions: We have identified that Smc haplosufficiency and AML1-ETO cooperate to promote increased self-renewal and immature cell morphology. Additionally, Smc3 haplosufficiency and AML1-ETO result in increased chromatin accessibility and transcriptional changes. Our findings lead us to propose that alteration of cohesion as a regulator of chromatin accessibility allows AML1-ETO to bind new sites leading to the transcriptional changes and downstream phenotype we have observed here. Significance: Our work provides insights into how we may begin to develop more personalized, targeted therapies for leukemias, based on the combination of specific driving mutations present in an individual patient (ie cohesion targeting therapies may be successfully used for patients with t(8;21) but not inv(16)).


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Title: Gammaherpesvirus aspers host IL-17 signaling to support chronic infection

Introduction: Gammaherpesviruses are oncogenic viruses that are associated with multiple cancers, including B cell lymphomas. These viruses have a natural tropism for B cells and drive a robust germinal center (GC) response, needed to establish a latent reservoir in memory B cells. The robust GC response driven by gammaherpesvirus infection increases the risk for B cell transformation. Unsurprisingly, many gammaherpesvirus cancers are derived from GC or post GC B cells. The mechanism used by gammaherpesviruses to induce the GC response is poorly understood.

IL-17 is a functionally diverse cytokine that has been found in almost every cancer type. Interestingly, herpesvirus saimiri (HVS), a simian gammaherpesvirus, encodes a viral IL-17, which functions like human IL-17A, suggesting an intriguing possibility that IL-17A is important in gammaherpesvirus infection. Other gammaherpesviruses do not encode a vIL-17 and therefore are not required to support the germinal center response during gammaherpesvirus infection. Further, neutralization of IL-17A during infection led to a decrease in viral latency and reactivation as well as attenuation of the germinal center response. Taken together these results establish a proalveolar role for IL-17A signaling during gammaherpesvirus infection.

Conclusions: IL-17A signaling supports the germinal center response and establishment of chronic infection during gammaherpesvirus infection. Significance: This study shows for the first time a proalveolar role of IL-17A signaling in an oncogenic virus infection.

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Basic
Title: Inhibition of HIF-35 Sensitive Primary and Metastatic Liver Cancer Cells

Introduction: Transarterial chemoembolization (TACE) is the standard of care for unresectable primary liver tumors. However, robust data demonstrating the superiority of chemoembolization over bland embolization is lacking. Recent studies have shown that embolization induces transient hypoxia which further drives proliferation of hypoxia-adapted cancer cells and leads to resistance to drugs.

Objective: The purpose of this study is to determine whether HIF-35 inhibition sensitizes cancer cells to doxorubicin and therefore can improve the outcomes of embolization.

Methods: Hepatocellular carcinoma (MCA-RH777), colorectal liver metastasis (CC-531) and immortalized liver (Clone 9) cell lines were grown in DMEM medium at 37°C in a humidified atmosphere with 5% CO2 in normoxic and 1% O2 hypoxic conditions. For survival assays, the cells were exposed to doxorubicin (drug used for TACE), R0949 (HIF-1α inhibitor) or a combination of the two under normoxic or hypoxic conditions. After 24h and 48 h of incubation, images were acquired and cell viability was determined by trypan blue staining. For wound healing assay, cells were seeded into 12-well plates to achieve confluency. The following day, a scratch wound was made, and the cells were cultured in medium and subjected to the above described treatments. Images of the cells migrating into the wound were captured prior to and after 16h.

Results: The combination treatment of doxorubicin and R0949 showed greater than 12.5% cell death under hypoxic conditions compared to normoxic conditions in the cell lines tested. Although cell death was noted with either treatment alone under hypoxic conditions, the combination was shown to be markedly effective (>30%). Similarly, combination therapy indicated greater inhibition of migration (>20%) across the wound edges under hypoxic conditions compared to normoxic conditions. Migration was inhibited in both treatment arms; however, combination therapy under hypoxia showed higher inhibition.

Conclusion: Combination therapy enhances the efficacy of doxorubicin and reduces tumor resistance under hypoxic conditions. Further investigations are underway to understand the cell cycle arrest in combination treatment contributing to the inhibition of cell proliferation. Significance: This result suggests that combination therapy-induced liver tumor sensitization and could be a promising step in transarterial therapy.
Introduction: SHC1 proteins exists in three functionally distinct isoforms (p46SHC, p52SHC, and p66SHC) that serve as intracellular adaptors for several key signaling pathways in breast cancer. Testing the isoform-specific roles of SHC1 proteins in breast cancer initiation and progression has been inaccessible due to the lack of isoform-specific inhibitors or gene knockout models.

Objective: To test the role of individual SHC1 isoforms in initiation and progression of breast cancer and identify novel targets for drug therapies.

Methods: Human breast tissue from the MCW Tissue Bank and Human INSTA-Blot™ Breast Tissue OncoPair ready-to-use PVDF membranes were used to assess total SHC proteins expressions in human breast cancer and normal tissue. Staining for ER, PR, and HER2 were performed using Dako Envision Flex™ mini kit. To test the role of individual SHC1 isoforms we have generated the first isoform-specific gene knockout models for p52SHC and p66SHC, using germline gene-editing in rat strain. Whole mount staining of rat mammary glands was used to analyze the difference in mammary gland structure between wild type (WT) and genetically modified rats. Rats were dosed with DMBA by oral gavage to induce mammary tumors, and progression of tumor development was followed for 15 weeks. At 15 weeks tumors were excised and analyzed by RNA-seq to determine differences between tumors lacking p66SHC or p52SHC.

Results: Expression of SHC1 proteins was found to be up-regulated in human breast carcinomas. The comparison of DMBA-induced rat tumors with normal rat mammary tissue also revealed increased expression of SHC1 isoforms. Compared with WT rats, genetic ablation of the p52SHC isoform significantly attenuated mammary tumor formation, whereas the p66SHC knockout had no effect. These data, combined with p52SHC being the predominant isoform that is upregulated in human and rat tumors, provide the first evidence that p52SHC is the oncogenic isoform of SHC1 gene that promotes breast cancer. Compared with WT tumors, p52SHCKO tumors displayed a differentially expressed genes with only 18 genes in the p66SHCKO tumors, further highlighting that p52SHC is the relevant SHC1 isoform in breast cancer. Finally, gene network analysis revealed that p52SHC KO disrupted multiple key pathways that have been previously implicated in breast cancer initiation and progression, including ESR1 and mTORC2/RICTOR.

Conclusions: Our data demonstrate that p52SHC isoform is the key driver of DMBA-induced breast cancer while the expression of p66SHC and p46SHC are not enough to compensate. Significance: Our data strongly suggest that p52SHC-dependent signaling is required for mammary tumor progression and suggest p52SHC may serve as node interfacing several critical signaling pathways implicated in breast cancer progression. Our finding that p52SHC appears to be a central hub regulating these pathways provides rationale for an exciting target for new drug therapies.

Confounder source: MCW Pilot Grant, NIH grants R01 DK098159 and grant from the Wisconsin Breast Cancer Showhouse, Inc. and the Medical College of Wisconsin Cancer Center.

Fundingsource: NIH R01 HL133029, We Care Foundation Grant; MCW-Cardiovascular Center Pre-PKG grant, Advancing a Healthier Wisconsin – Redox Biology Grant.
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<th>Schlaak, Rachel</th>
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<th>Basic</th>
<th>Identifying Hereditary Factors That Modulate Radiation-Induced Cardiac Toxicity</th>
<th>MCW Cancer Center Pilot Grant; Michael H. Keelan, Jr., MD, Foundation Grant; Mary Kay Foundation Award Grant No. 017-29; Nancy Lanning Sobczak, PhD, Breast Cancer Research Award; Susan G. Komen, American Cancer Society Institutional Research Grant 86-004-26; and Grant BK12TR000056.</th>
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**Introduction:** Over half of all cancer patients receive radiation therapy (RT) as part of their cancer treatment. Radiation can improve cancer outcomes, but toxic normal tissue side effects may occur causing morbidity and mortality. Radiation-induced cardiac toxicity has been reported in patients with thoracic tumors, such as lung and breast cancers. However, there are currently no biomarkers or protective agents to predict or protect against radiation-induced cardiotoxicity.

**Objective:** The purpose of this study was to use animal models to assess RT cardiac toxicity, followed by genetic mapping of radiation sensitivity, to identify new biomarkers that predict cardiac toxicity from RT.

**Methods:** Salt-sensitive (SS) and Brown Norway (BN) rats were selectively bred to produce SS.BN3 consomic rats, which are genetically identical to SS rats except chromosome 3 is inherited from the BN rat. We have previously shown that SS rats exhibit enhanced cardiac toxicity compared to SS.BN3 rats (Schlaak et al., 2019). Congenic mapping was used to identify regions on chromosome 3 modifying radiation sensitivity. Adult female SS, SS.BN3, and congenic rats (CG.1, CG.2, CG.3 and CG.4) received image-guided localized whole-heart RT of 24 Gy (AP and 2 lateral fields, weighted 1:1:1). Echocardiograms with strain analysis were performed at baseline, and 3 and 5 months. Also, RNA-seq with IPA analysis and immunohistochemistry was performed on SS and SS.BN3 left ventricle tissue.

**Results:** Congenic rat strain CG.4 exhibited enhanced cardiac toxicity compared to the SS.BN3 rats. At 5 months post-RT, only the SS and CG.4 rats displayed cardiac hypertrophy (p<0.01). The chromosomal region unique to the SS and CG.4 rat strain is 25 MB, which displays enhanced radiation sensitivity compared to SS.BN3. RNA-seq gene expression analysis demonstrated mitochondrial dysfunction genes were significantly increased in SS vs BN3. 331 genes are located in the 25 MB region of interest, with 93 genes (>0.9% of genome) differentially expressed. Immunohistochemistry also showed increased mast cells in the SS.BN3 left ventricle tissue at 10 weeks post-RT compared to SS (p<0.05).

**Conclusions:** Similar to SS rats, the CG.4 rats are more sensitive to cardiac radiation than the SS.BN3 rats, indicating a role for heritable factors in determining radiosensitivity. Gene expression analysis showed numerous potential targets on the 25 MB region on chromosome 3, including genes involved in mitochondrial dysfunction, sirtuin signaling, notch signaling, and apoptosis. The increase of mast cells in SS.BN3 left ventricle tissues compared to SS tissue may also be contributing to the observed differences in heart damage.

**Significance:** Genetic mapping will aid in narrowing the causative target(s) to improve potential therapies. This project has the potential to enhance the effectiveness and toxicity profile of RT in cancer.

**Funding source:** MCW Cancer Center Pilot Grant; Michael H. Keelan, Jr., MD, Foundation Grant; Mary Kay Foundation Award Grant No. 017-29; Nancy Lanning Sobczak, PhD, Breast Cancer Research Award; Susan G. Komen, American Cancer Society Institutional Research Grant 86-004-26; and Grant BK12TR000056.

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<th>Sun, Yunguang, MD, PhD</th>
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<th>Rui, Hallgeir, MD, PhD</th>
<th>Basic</th>
<th>NSG-Pro: a next generation model for breast cancer patient-derived xenografts</th>
<th>NIH/NCI R01CA188575, MCW ACS-IRG</th>
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**Introduction:** Patient-derived xenograft (PDX) models of breast cancer have existed since 1975, yet the inability to grow human estrogen receptor (ER) breast tumors in rodent hosts precluded studying the majority of patients that ultimately progress to lethal metastatic disease. We demonstrate that the failure of current PDX hosts is due to the inability of mouse prolactin (Prl) to cross-react with the human prolactin receptor (hPrlR), a required endocrine factor for the survival, growth, and metastatic progression of ER+/PR+ PDX.

**Objective:** We hypothesize that the lack of hPrl in the mouse PDX host strain deprivates human ER+/luminal breast cancers of a necessary endocrine factor that supports breast tumor engraftment, growth, metastasis, and therapeutic response. Notably, introducing hPrl into a PDX host strain would be predicted to have little or no negative impact on ER-/PR- or HER2+ breast PDX biology, suggesting that a humanized-prolactin PDX host strain increase engraftment of luminal ER+/breast cancer and maintain the high engraftment rates of other PDX subtypes.

**Methods:** We engineered NSG-Pro mice by introducing hPrl1 to host NSG mice. These mice were used to implant clinically dissected human breast cancer tissue. Transplantable PDX lines were studied with drug response experiments such as Tamoxifen, Parp1 inhibitor or Her2 humanized antibody Trastuzumab. Also, RNA seq and WGS were performed to explore the pathways and mutations contributing to Tamoxifen resistance.

**Results:** NSG-Pro mice showed >50% engraftment rates for ER+ and ER− PDX. These transplantable PDX models recapitulate the clinical features of its corresponding human breast cancer. RNA seq and WGS analysis revealed elevated Her2 and Parp1 in Tamoxifen-resistant sublines may be potential Tamarxifen resistance mechanisms. Targeting Parp1/Lgvs greatly inhibited resistance tumor growth. Trastuzumab blocks the growth of resistance tumors effectively but drugs resistance eventually develops.

**Conclusions:** In summary, our study showed that NSG-Pro-mice was the first PDX platform with >50% engraftment rates for ER+ and ER− PDX. These transplantable PDX models developed recapitulate the clinical features of its corresponding human breast cancer. RNA seq and WGS analysis revealed elevated Her2 and Parp1 in Tamoxifen-resistant sublines may be potential Tamarxifen resistance mechanisms. Targeting Parp1/Lgvs greatly inhibited resistance tumor growth. Trastuzumab blocks the growth of resistance tumors effectively but drugs resistance eventually develops.

**Significance:** NSG-Pro mice provide powerful patient avatars for precision medicine to identify molecular vulnerabilities and interrogate biology and drug responsiveness of ER-positive human breast cancers.

**Funding source:** NIH R01, Cancer Center ACS seed grant
Introduction: Androgen deprivation remains the cornerstone for the management of advanced prostate cancer (PC). The second-generation anti-androgen, enzalutamide (ENZ), is FDA-approved for castrate-resistant (CR) PC and targets androgen receptor (AR) activity in CRPC. Despite initial clinical activity, acquired resistance to ENZ arises rapidly and most patients develop terminal disease. Stat5a/b (Stat5), which is both a signaling protein and a nuclear transcription factor is known to sustain viability of PC cells and to induce growth of PC xenograft tumors in vivo. Blockade of Stat5 signaling induces apoptotic death of PC cells, suppresses growth of both xenografted and autochthonous PC tumors as well as clinical patient-derived PCs ex vivo in culture. Conversely, overexpression of active Stat5 has been shown to induce proliferation of PC cells in vitro and growth of PC tumors in mice. Stat5 induces metastatic progression of PC, as evidenced by Stat5's promotion of metastasis formation in vivo, and induction of hallmarks of EMF and stem-like cancer cell properties through induction of Twist1 and BMI1 expression in PC. In 30-40% of advanced CRPCs, the chromosome 17 locus encompassing STAT5A and STAT5B genes undergoes amplification resulting in increased Stat5 protein levels. Notably, high nuclear Stat5 protein expression at the time of the initial PC treatment predicted recurrence of the disease in three independent cohorts totaling 1,035 patients. The predictive role of active Stat5 for clinical PC progression to lethal CR state corroborates involvement of Stat5 in PC progression in preclinical PC models. The findings of Stat5 as a PC growth promoter and a predictor of PC recurrence imply involvement of Stat5 in development of CRPC, which led us to hypothesize that Jak2‐Stat5 signaling sustains viability of PC cells following disruption of AR signaling by ENZ.

Objective: To investigate the significance of Jak2‐Stat5 signaling in resistance of PC to ENZ.

Methods: Levels of Jak2 and Stat5 activation were evaluated by immunohistochemistry, immunoprecipitation and western blotting in PC cells, xenograft tumors and in patient-derived PCs ex vivo in 3D tumor explant culture. Jak2 and Stat5 were suppressed either by genetic knockdown using lentiviral expression of shRNA or pharmacologically by Jak2 and Stat5 inhibitors. Levels of mRNA were assessed by QPCR and gene expression profiling. A unique PC xenograft mouse model (CWR22Pc), which mimics PC clinical progression in patients, was utilized to assess in vivo responsiveness of primary and ENZ resistant PCs to inhibition of Jak2/Stat5 as a second-line treatment. Patient-derived clinical PCs were tested for responsiveness to bromodomain blockage ex vivo in tumor explant cultures.

Results: We demonstrate, for the first time, that ENZ induces a robust increase in Stat5s activation in PC cells in vitro, in xenograft tumors in vivo and in patient-derived PCs during ENZ treatment. Mechanistically, ENZ‐liganded AR induced rapid and sustained Jak2 phosphorylation in PC cells through a process involving Jak2‐specific phosphatases PTPΕ and SHP2. ENZ‐induced Jak2 activation leads to Stat5 phosphorylation, and a formation of a feed-forward loop in PC cells, where active Stat5 increases Jak2 mRNA and protein levels. We further demonstrate that activated Stat5 promotes growth of PC cells during ENZ treatment and, at the same time, inhibition of Stat5 as a second-line treatment induces extensive death of PC cells surviving ENZ treatment. Pharmacological Stat5 blockade inhibited castrate resistant growth of PC xenograft tumors after ENZ resistance developed and induced further death after ENZ treatment in patient‐derived PCs ex vivo in tumor explant cultures. Mechanistically, residual PC cells which survived genetic knockdown of AR could be eliminated by suppression of Stat5, implying a presence of Stat5‐regulated, AR independent growth mechanisms. In summary, this work supports the novel concept of a critical role of a hyperactive Jak2‐Stat5 signaling loop in promoting resistance of PC to ENZ. Pharmacological Jak2‐Stat5 inhibition may provide an effective therapy for Stat5‐positive advanced PC in combination with ENZ or after ENZ fails. Conclusions: ENZ‐liganded AR induces sustained Jak2 and Stat5 phosphorylation in PC leading to a formation of a positive feedback loop, where activated Stat5, in turn, induces Jak2 mRNA and protein levels contributing to further Jak2 activation. Collectively, this work introduces the novel concept of a pivotal role of Jak2/Stat5 signaling in mediating resistance of PC to ENZ which represents a readily targetable molecular vulnerability in ENZ‐resistant Stat5‐positive PCs.
| Yeo, Chay Teng | Graduate Student | Corbett, John, PhD | Basic | The regulation of oxidative metabolism by nitric oxide protects β-cells from DNA damage-induced cell death | NIH/NIDDK-R01DK052194, NIH/NAID R01 AI044458 and a gift from the Forest County Potawatomi Foundation. |

**Introduction:** Nitric oxide, generated in β-cells in response to cytokines, induces DNA damage that activates DNA damage response (DDR) and eventually leads to apoptosis if damage is too extensive. We showed that nitric oxide, while inducing double-strand DNA breaks (DSBs), also inhibits DDR signaling and protects β-cells from DNA damage-induced apoptosis. The ability of nitric oxide to inhibit the DDR signaling is selective to β-cells and correlates with a loss in ATP levels, while non-β-cells are able to maintain both ATP levels and DDR signaling when treated with nitric oxide.

**Objective:** We tested the hypothesis that β-cell metabolism enables nitric oxide-dependent inhibition of DDR and protection from DNA damage-induced cell death.

**Methods:** INS 832/13, MEFs, and HepG2 were cultured in a full media supplemented with 10% FBS. Proteins were extracted by lysing cells in laemmli buffer, and were determined via Western blot. Nucleotides (ATP and NAD+) were extracted from treated cells by perchloric acid precipitation and quantified by HPLC analysis. Metabolomics analysis was performed to determine change in metabolites from treated cells harvested in 80% methanol containing C13-labelled internal standards, and measured via a 1200 Infinity Series HPLC in-line with a 6430 QqQ mass spectrometer. Extracellular flux analysis (Oxygen consumption rate and extracellular acidification rate) was performed on a Seahorse XF96 analyzer. Cell death was determined by measuring incorporation of the fluorescent SYTOX Green nucleic acid stain.

**Results:** β-cell metabolism is characterized by the tight coupling of glycolysis to mitochondrial oxidative metabolism. Nitric oxide is known to inhibit mitochondrial oxidative metabolism (e.g., aconitase in TCA cycle and complex IV of electron transport chain (ETC)). Under inhibition of mitochondrial oxidation by nitric oxide, β-cells are not able to compensate for their loss in ATP by increasing glycolytic metabolism. In contrast, non-β-cells compensate for the mitochondrial inhibition by increasing glycolytic metabolism to maintain their ATP pool. Consistent with the action of nitric oxide, we showed that rotenone, an inhibitor of complex I of ETC, also inhibit DDR signaling and decrease ATP levels in β-cells but not in non-β-cells. Importantly, when non-β-cells are forced to generate ATP via mitochondria, nitric oxide inhibits DDR signaling, decrease ATP levels, and protects cells from DNA damage-induced apoptosis.

**Conclusions:** Our findings suggest that the tight coupling of glycolysis to mitochondrial oxidative metabolism in β-cells allows nitric oxide to decrease ATP levels and subsequently inhibit DDR signaling, and eventually protects β-cells from DNA damage-induced apoptosis.

**Significance:** Targeting DDR signaling in cancer cells is exploited for cancer treatment. These findings may provide mechanistic insights into the potential role of intermediary metabolism as a contributor to the efficacy of DNA damaging agents in killing cancer cells.

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