THE PENNSYLVANIA STATE UNIVERSITY
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ARYL HYDROCARBON RECEPTOR ANTAGONISM ATTENUATES INFLAMMATORY AND GROWTH FACTOR GENE EXPRESSION IN FIBROBLAST LIKE SYNOVIOCYTES FROM INDIVIDUALS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune disease affecting the synovium of joints. The disease is characterized by chronic innate and adaptive inflammatory signaling, mediated at least in part by a number of pro-inflammatory cytokines including interleukin 1 beta (IL1B). The genes IL1B and cyclooxygenase 2 are upregulated in this proinflammatory environment and create a positive feedback-signaling loop between synoviocytes and immune cells. In addition, vascular growth factor A, which is induced during inflammation and contributes to invasive potential of synoviocytes into bone as the rheumatoid arthritis process progresses. The role of the Aryl hydrocarbon receptor (AHR) in IL1B-mediated expression of pro-inflammatory and mitogenic genes associated with rheumatoid arthritis pathology was examined.

Results revealed that AHR antagonism with the compound GNF-351 attenuates pro-inflammatory gene expression in primary fibroblast like synoviocytes from individuals with rheumatoid arthritis. Using qPCR ,siRNA, knockdown of AHR, and ELISA results revealed that GNF-351 exerts its effects through AHR antagonism and represses both mRNA levels and secreted protein levels of a number of pro-inflammatory genes. This study shows that AHR antagonism is a potential approach to treat rheumatoid arthritis induced inflammation.

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Chapter 1

Introduction

Aryl Hydrocarbon Receptor

The aryl hydrocarbon receptor (AHR) is a soluble receptor in the basic helix-loop-helix/PER-ARNT-SIM superfamily of genes.[1] The AHR is a ligand-activated transcription factor involved in xenobiotic metabolism, particularly the metabolism of polycyclic aromatic hydrocarbons (PAHs) as well as various endogenous compounds.[2] The AHR plays an important, yet poorly understood, role in development. AHR knockout models in mice have shown that the AHR is important for reproduction, the vascular development in the liver, and immune system function.[3] AHR in the adult seem to be important for adaptation to environment including diet, chemical insult, and changing oxygen levels.[4,5]

Inactive AHR is typically found in the cytosol bound to chaperones HSP90, X-associated protein 2, and p23.[6] Upon ligand binding, the AHR translocates to the nucleus, dissociates with chaperones and heterodimerizes with its binding partner, the aryl hydrocarbon receptor nuclear translocator (ARNT). The activated AHR/ARNT complex binds to dioxin response elements (DREs) with the sequence 5′-TTGCGTGAGAA-3′ in the promoter regions of a wide array of genes, regulating gene expression.[7] The AHR is also capable of interacting with other signaling pathways, such

as estrogen receptor, peroxisome proliferator activator receptor, and NF-κB creating cross-talk between these pathways.[6,8,9]

The classic AHR agonist is 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) whose toxicity is mediated by AHR-driven alterations in gene expression.[10] Target genes of AHR express proteins involved in xenobiotic metabolism such as the cytochrome P4501A1, glutathione-s-transferase, and glucuronyl-transferase.[11,12] Genes in inflammatory signaling are also upregulated, including interleukin 1 beta (IL1B) and interleukin 6 (IL6) by the AHR.[13] The AHR has also been shown to modulate expression of genes affecting cell cycle such as growth factors like epiregulin[14], this AHR activity can be inhibited through the use of competitive antagonists such as GNF351. These compounds bind the AHR ligand binding site, but do not activate AHR mediated transcriptional activity. In this study, GNF-351 is used to demonstrate that AHR antagonism attenuates constitutive and inducible AHR-mediated gene expression.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a progressive autoimmune disease that affects approximately 1% of the population.[15] It is characterized by chronic inflammation of the synovium, the lining of the joint, particularly in smaller joints in the feet and hands. The synovium is a porous network of extracellular matrix proteins, collagen, and two types of synoviocytes, macrophage like synoviocytes and fibroblast like synoviocytes (FLS).[16] The synovium normally provides growth and nutrition factors as well as lubrication to the joint.

In the pro-inflammatory environment of RA, FLS cells adopt a tumor-like phenotype, releasing pro-inflammatory mediators and becoming hyperplastic.[17] Like transformed tumor cells, RA-FLS have increased migratory and invasive potential. The synovium, which is usually only 1-2 cells thick, can become as much as 20 cells thick with increased numbers of synoviocytes, as well as leukocytes that have been recruited to the joint and is referred to as a pannus.[16] The cells of the pannus then produce large numbers of proteins involved in inflammation, pain, migration, invasiveness, and tissue remodeling including interleukin 1 β (IL1B), interleukin 6 (IL6), tumor necrosis factor alpha (TNFA), chemokine C-C motif ligand 20 (CCL20), and cyclooxygenase 2 (COX2), vascular endothelial growth factor-A (VEGFA), fibroblast growth factor (FGF), epiregulin (EREG), amphiregulin (AREG), and matrix metalloproteinases (MMPs) 2 and 9.[16,18-21] These mediators of inflammation, cytokines, and enzymes promote recruitment and activation of other leukocytes, further release of pro-inflammatory mediators, angiogenesis, and destruction of both cartilage and bone through modification of osteoblasts and osteoclasts. In severe cases of RA, pro-inflammatory cytokines can produce systemic inflammation and other effects on multiple organ systems.

Epidemiologic studies have shown a significant positive correlation between smoking and the development and aggressiveness of RA.[22] Tobacco smoke is a source of a number of AHR agonists as a result of combustion. Also, reports have shown that synovial fluid from RA patients have elevated levels of kynurenic acid (KA), an endogenous AHR ligand.[23] Furthermore, it has been shown that a combination of an AHR agonist and pro-inflammatory cytokine such as IL6 can synergistically up-regulate expression of target genes.[24]

Interleukin 1 Beta

IL1B is a pro-inflammatory cytokine produced by a wide variety of cell types in response to various stimuli including pathogen associated molecular patterns, danger associated molecular pattern, and by transcription factor activity such as NF-κB and AHR.[25] The cytokine is involved in a number of cellular pathways including proliferation, apoptosis, inflammation. IL1B is first produced as a 31 kDa pro-protein that is subsequently cleaved by Caspase-1 into the active protein. The active protein binds to the IL1B receptor and stimulates production of a number of pro-inflammatory cytokines including TNFa, IL6, and IL1B itself.[25]

IL1B is of particular interest in RA as it also stimulates the production of members of the endothelial growth factor family, which increases the invasive capabilities of RA, and has been shown to be the major mediator of these properties in the disease. Production of MMPs including collagenases and stromelysins which ultimately degrade connective tissue in the joint, are also stimulated by IL1B. Additionally, IL1B is a potent stimulator for osteoprotegerin-ligand (OPG-L). OPG-L is the crucial signal for osteoclast differentiation and activation, which are responsible for degradation of bone seen in severe arthritis.[26]

Cyclooxygenase-2

COX2, also known as Prostaglandin-endoperoxide Synthase-2 (PTGS-2), is a homo-dimeric enzyme that helps catalyze the conversion of arachidonic acid to prostaglandin H2 (PGH2).[27] PGH2 is the precursor for a number of biologically active

molecules including thromboxanes, prostacyclins, and other prostaglandins. These molecules are involved in hemostasis, and stimulating inflammation and hyperalgesia which are clinical manifestations of RA in the joint.[27]

Vascular Endothelial Growth Factor A

VEGF is a potent mitogen, and is involved in angiogenesis in response to hypoxia via HIF1a signaling.[28] In RA, hypoxia is induced by synovium hyperplasia and expansion. The existing vasculature is not sufficient for the growing and dividing synovium. Angiogenic factors like VEGFA are released by synoviocytes stimulating the formation of new blood vessels.[29] As is the case in many cancers, this is a poor prognostic feature of the disease. High levels of VEGFA and angiogenesis create an almost malignant phenotype allowing for the invasion of surrounding tissues.[30]

Hypothesis and Goals

Correlations between smoking and RA, as well as elevated levels of genes and cytokines such as COX2 and IL6 that have been previously shown to be regulated by the AHR led to the hypothesis that the AHR is also involved in the expression of COX2 as well as IL1B and growth factors like VEGFA in FLS cells in RA.[31] The AHR has been shown to mediate expression of IL6 through binding to dioxin responsive elements 3 kb upstream of the transcriptional start site and synergizing with inflammatory signals.[24] As mentioned previously, FLS-RA cells share a similar phenotype with transformed cancer

cells. FLS cells become hyperplastic and have increased migratory and invasive potential in the pannus.

The goal of this study is to demonstrate that the AHR mediates expression of cytokines and growth factors that ultimately lead to joint inflammation and destruction in RA. GNF-351 is used to show that antagonism of the AHR can attenuate expression of these genes in a pro-inflammatory environment, representing a novel therapeutic approach to the treatment of RA. Treatment of FLS-RA cells with IL1B is used to replicate this environment in vitro. siRNA knockdown of AHR is performed to confirm that repression in gene expression with GNF-351 is AHR dependent. Enzyme linked immunosorbent assays are used to confirm results from qPCR analysis of gene expression.

Chapter 2

Materials and Methods

Cell Culture:

Primary human fibroblasts like synoviocytes were obtained from individuals with rheumatoid arthritis in cooperation with the Hershey Medical Center. FLS cells maintained in synoviocyte growth medium and supplemented with fetal bovine serum, penicillin, and streptomycin. Cells were stored at 37°C and 5% CO_2. All experiments were carried out at the fourth doubling time.

Lactate Dehydrogenase Assay:

FLS were treated with increasing concentrations of GNF 351(DMSO vehicle control 200 nM GNF351, 500 nM GNF 351, or 1 μM GNF351) every 12 h over a 48 h period. 48 h post-treatment, whole cell lysate and culture media were analyzed for LDH. Lactate dehydrogenase-based in vitro toxicology assay kit (Sigma Aldrich, St. Louis, Missouri, USA) was used per manufacturer's instructions. Each treatment was performed in triplicate and the average with standard error is shown.

RNA Isolation and Reverse Transcription:

Total RNA was isolated from primary HFLS cells using TRIReagant from Sigma Aldrich as specified by the manufacturer. RNA was diluted to the appropriate

concentration and cDNA was prepared using ABI high-capacity cDNA archive kit (Applied Biosystems).

Real time PCR:

Gene expression was measured using quantitative real time PCR using PerfeCTa SYBR green reagant from Quanta in a 25 µL reaction. MyiQ single color real-time PCR detection system was used for data analysis (Bio-RAD, Hercules, CA). Expression of mRNA was normalized to expression of L-13a. Results were graphed using GraphPad Prism 5. Experiments were performed in triplicate with average and standard error shown. Statistical significance was calculated using one-way ANOVA. Significance is noted as *= $p<0.05$, **= $p<0.01$, ***=$p<0.001$.

Gene Silencing:

Small interfering RNA (siRNA)-mediated AHR knockdown in HFLS-RA cells was performed by Dharmacon siRNA (control oligo D-001810-0X, AHR oligo J-004990-07) using the U-020 programme of Amaxa nucleofection system (Lonza, Walkersville, Maryland, USA). $2x10^6$ cells were electroporated and transfected with siRNAs targeted at AHR mRNA. siRNA-transfected cells were seeded into 6-well plates at 2 ml/well synoviocyte growth media. Cells were cultured for 48 h post-transfection. Verification of AHR knockdown was achieved through western blot analysis.

Western Blot Analysis:

Whole cell extracts were prepared and resolved on 8% Tricine sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) gels, and detected with protein specific antibodies. Specific proteins were visualised with biotin-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, Pennsylvania, USA) in conjunction with ^{125}I-streptavidin. Radioactivity levels were quantified by excision of protein bands and through the use of a γ counter.

Enzyme Linked Immunosorbent Assay:

For measurement of secretory cytokine levels, HFLS-RA cells were treated with GNF-351 followed by stimulation with 10 ng/ml IL1B for IL6 secretion and with 100 ng/ml lipopolysaccharide (LPS) for IL1B secretion. Cell supernatants were stored at −80°C. In order to determine the levels of secretory cytokines, IL1B and IL6, ELISA was performed per manufacturer's instructions (BioLegend). Experiments were performed in triplicate and the mean and standard error of the mean are shown.

Chapter 3
Results and Discussion

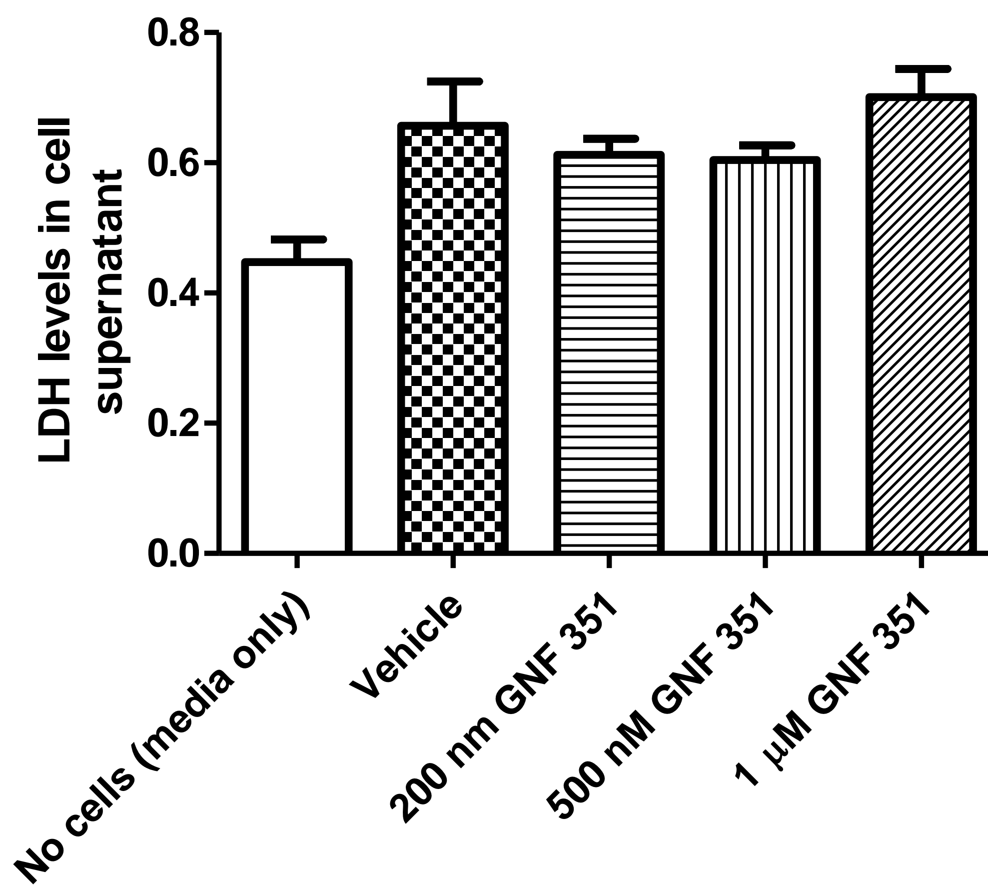

Figure 1. Lactate Dehydrogenase Assay of primary FLS-RA cell supernatant. Primary FLS-RA cells were treated every 12h with increasing concentrations of GNF-351 over a 48h period then supernatant was assayed for LDH 48h post-treatment.

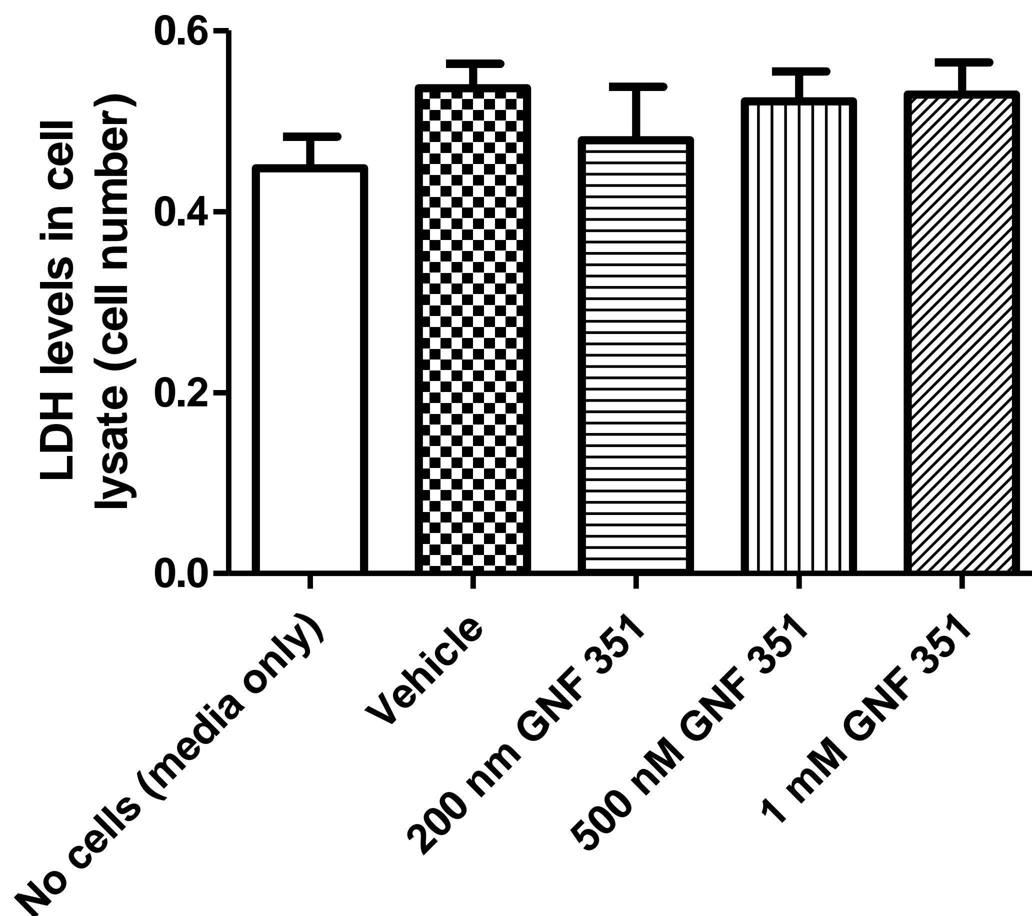

Figure 2. Lactate Dehydrogenase Assay of primary FLS-RA cell lysate. Cells were again treated every 12h with increasing concentrations of GNF-351 over 48h. Cells were lysed and assayed for LDH 48h post-treatment.

Lactate Dehydrogenase Assay

Lactate dehydrogenase is released by cells in response to cellular injury. A dose response LDH assay was performed in order to assess the toxicity of GNF-351 in FLS cells from 200 nM to 1 μM. Increasing levels of LDH indicate increased cytotoxicity. A slight increase in LDH levels was observed with vehicle and GNF-351 treatments relative to media only, however, these changes were not significant. There was no significant change in LDH levels with increasing doses of GNF-351 relative to vehicle control in both cell supernatant (figure 1) and cell lysate (figure 2). LDH was neither released by the cell in significantly increased amounts, nor was it produced intra-cellularly at higher levels. This indicated that GNF-351 was not toxic to HFLS-RA cells at doses up to 1 μM.

Cytotoxicity is an important factor consider not only for this experiment, but also the ultimate goal of drug design and therapy. The LDH assay shows that GNF-351 is not toxic to FLS-RA cells at the doses used in this experiment implying that changes in gene expression are not due to a decreased number of cells as a result of GNF-351 toxicity. Toxicity is also a major hurdle in drug development. While this toxicity screen is limited both in dose and cell type, it does not eliminate GNF-351 from possible candidacy for drug development.

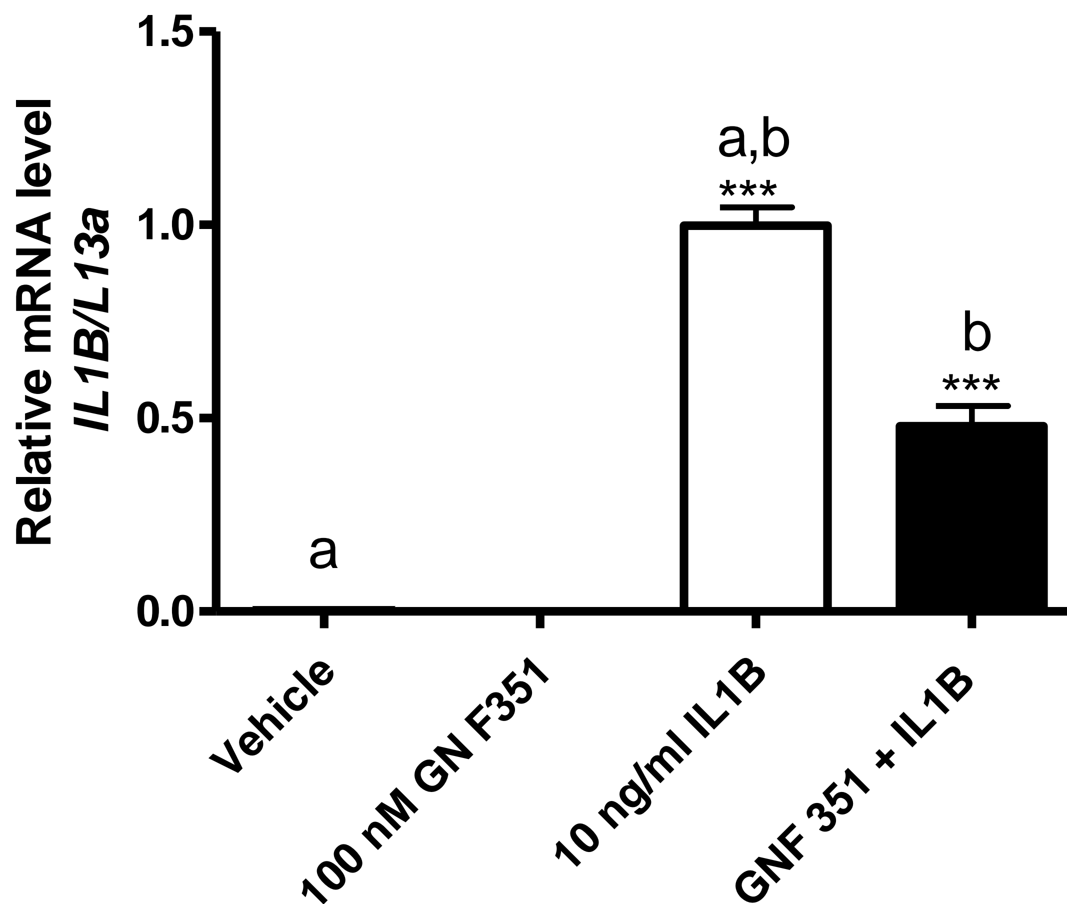

Figure 3. qPCR for IL1B expression in GNF351 and IL1B pretreated FLS-RA cells. HFLS-RA cells were pretreated with 100 nM GNF351 for 1h and then challenged with 10 ng/mL IL1B for 4h. Total cell lysate was collected and total mRNA isolated and converted to cDNA. qPCR was performed to analyze IL1B expression. Values were normalized to L13a.

Real Time PCR

Changes in gene expression patterns were assessed using quantitative RT-PCR. Expression of IL1B, and COX2 were examined. HFLS-RA cells were treated with 100 nM GNF-351, and then treated with 10 ng/mL IL1B for 4 hours. mRNA levels were normalized to L13a levels. Treatment with IL1B caused a significant induction in IL1B mRNA levels. Treatment with 100 nM GNF 351 caused no increase in IL1B expression. However pre-treatment with GNF-351 followed by IL1B stimulation caused an approximately 40% decrease in IL1B mRNA levels compared to IL1B treatment alone.

Even in the absence of an exogenous agonist, IL1B stimulates AHR mediated gene expression. GNF-351 was able to significantly reduce IL1B expression, as predicted. This repression was not 100% however. This indicates that either AHR was not completely antagonized by the treatment, it is still able to regulate gene expression in the presence of antagonist, or that there is another pathway regulating IL1B expression. While the former explanations may play some small role in the observed expression pattern, it is likely that pathways like NF-κB are co-regulating the gene.

Reduction in IL1B levels could cause a significant change of disease symptoms and pathology in vivo. As is demonstrated in the experiments, IL1B causes increased expression of IL1B, COX2 and a number of other pro-inflammatory mediators that are elevated in RA. Stopping this positive feedback loop is critical to the prevention of pathologic conditions in vivo.

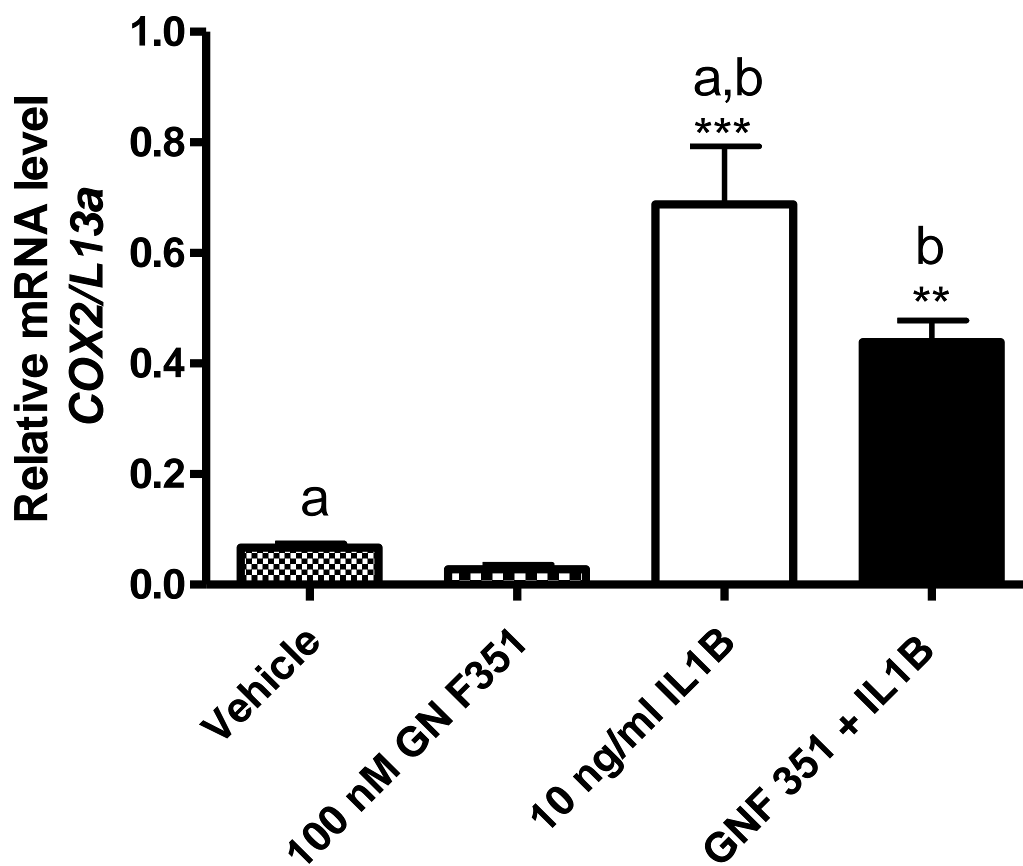

Figure 4. qPCR for COX2 expression in GNF351 and IL1B pretreated FLS-RA cells. HFLS-RA cells were pretreated with 100 nM GNF351 for 1h and then challenged with 10 ng/mL IL1B for 4h. Total cell lysate was collected and total mRNA isolated and converted to cDNA. qPCR was performed to quantify COX2 expression. Values were normalized to L13a.

The expression of COX2 was also examined (Figure 4). Treatment with 100 nM GNF 351 caused a slight decrease in COX2. Stimulation with IL1B caused an approximately 7-fold increase in COX2 mRNA compared to the vehicle treatment. Addition of GNF-351 attenuated this increase, causing an approximately 40% reduction in mRNA levels compared to IL1B treatment.. Attenuated expression of these genes concordant with GNF 351 treatment indicates that AHR is a mediator of expression.

Reduced expression of COX2 could have a significant impact on disease status in vivo. Increased levels off the enzyme in the synovium could lead to increased production of prostaglandins and prostacyclins, contributing to joint pain and inflammation. Treatment of these symptoms is already in practice through the use of steroids and NSAIDS. While many of these treatments only have one target such as a specific COX enzyme, or cytokine, AHR antagonism appears to attenuate the expression of a number of pro-inflammatory genes involved in RA.

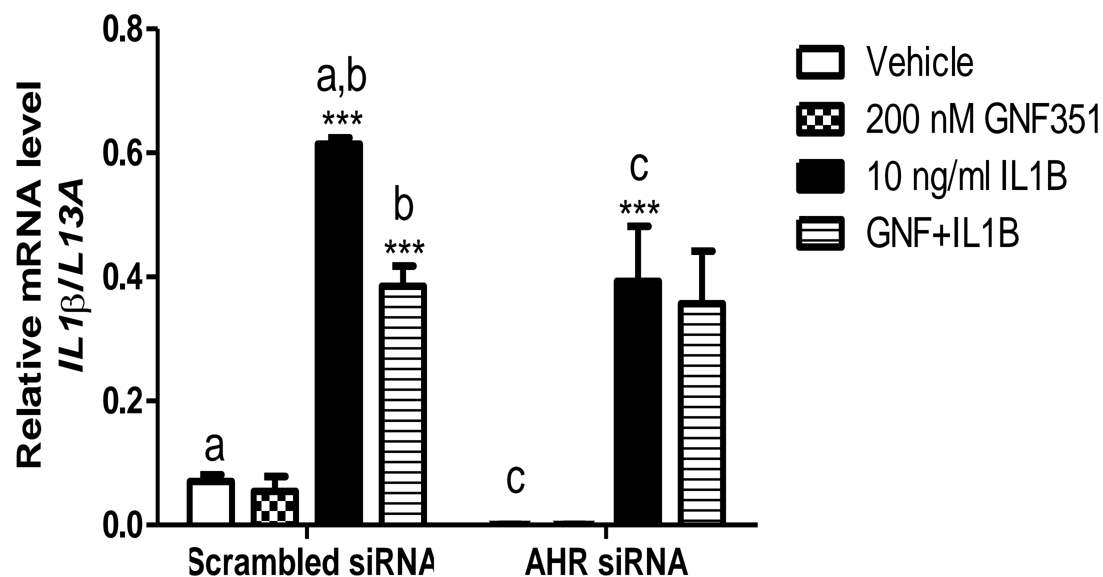

Figure 5. Small interfering RNA (siRNA)-mediated AHR gene ablation was performed in 2x106 primary FLS-RA cells per gene knockdown. AHR protein ablation was confirmed by western blot analysis of primary FLS-RA cells transfected with AHR siRNA for 48 h. Upon confir confirmation, primary FLS-RA cells transfected with AHR siRNA for 48 h were pretreated with 100 nM GNF-351 for 1 h followed by 10 ng/ml IL1B for 4 h. q PCR was used to quantify IL1B mRNA. mRNA expression was normalized to L13a expression.

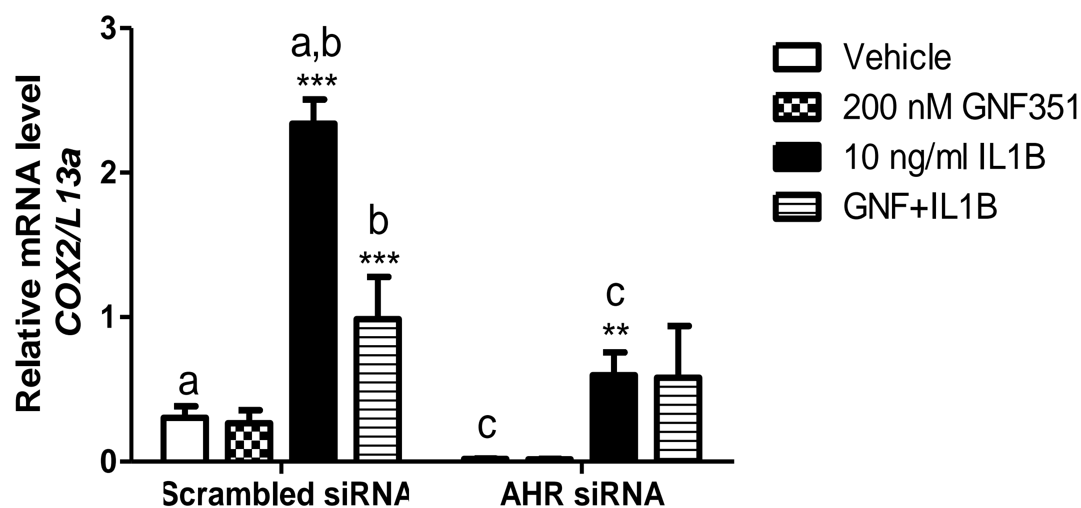

Figure 6. Small interfering RNA (siRNA)-mediated AHR gene ablation was performed in 2x106 primary FLS-RA cells per gene knockdown. AHR protein ablation was confirmed by western blot analysis of primary FLS-RA cells transfected with AHR siRNA for 48 h. Upon confir mation, primary FLS-RA cells transfected with AHR siRNA for 48 h were pretreated with 100 nM GNF-351 for 1 h followed by 10 ng/ml IL1B for 4 h. q PCR was used to quantify COX2 mRNA. mRNA expression was normalized to L13a expression.

siRNA AHR Gene Ablation

In order to confirm that reduction in cytokine and growth factor levels was mediated through AHR antagonism, siRNA knockdown of AHR was performed, and qPCR was carried out following IL1B stimulation and GNF-351 treatment. A western blot for AHR showed that siRNA knockdown of AHR was successful. Effect of AHR gene silencing was first examined on IL1B expression (figure 5). Treatment with scrambled siRNA showed similar results with the qPCR carried out previously. Stimulation with IL1B resulted in approximately 10 fold induction of IL1B induction. Treatment with GNF-351 significantly reduced the induction of IL1B. AHR knockdown resulted in overall decreased levels of IL1B expression. Treatment with IL1B caused an approximately 500 fold increase in IL1B expression. However treatment with GNF-351 failed to attenuate IL1B expression indicating that IL1B induced expression is at least partly mediated through the AHR.

Next COX2 expression was examined in response to AHR knockdown (figure 6). Similar to IL1B expression, COX2 expression is increase approximately 7-fold with IL1B stimulation relative to vehicle control and subsequently attenuated with GNF 351 treatment following addition of scrambled siRNA. After AHR knockdown, COX2 expression increases approximately 27 –fold following IL1B stimulation compared to vehicle treatment. GNF-351 treatment was unable to attenuate expression of COX2.

Decreased expression of IL1B and COX2 compared to scrambled siRNA treatment following AHR knockdown and stimulation with IL1B indicates that the AHR is involved in the regulation of both genes. GNF-351 treatment was unable to repress

IL1B and COX2 expression following AHR knockdown. This confirms that GNF-351 effects are mediated by the AHR and that these genes are regulated by AHR as hypothesized.

Attenuated levels of both IL1B and COX2 with only vehicle and GNF351 treatments following AHR knockdown suggests that the receptor has some constituitive activity in FLS-RA cells. The data suggests that the even in the absence of exongenous ligands or inflammatory stimulation, the AHR is able to drive gene expression of pro-inflammatory genes. This may play an important role in disease progression. These cells may be pre-disposed to inflammatory gene production, even before rheumatoid arthritis autoimmune reactions occur. This may also cause these cells to be hypersensitive to cytokine stimulation throughout the course of the disease.

While the conventional mechanism of regulation involves AHR agonist binding, translocation, heterodimerization with ARNT, and binding to DREs, it is unclear whether or not this is the mechanism in this scenario. IL1B has not been shown to be a direct AHR agonist, but has been shown to synergistically potentiate AHR mediated activity. The mechanism of this interaction is unclear. IL1B may act to potentiate the effects of AHR activated by endogenous agonists, or may activate AHR in a novel fashion. This stimulation of AHR gene regulation can be repressed through AHR antagonism.

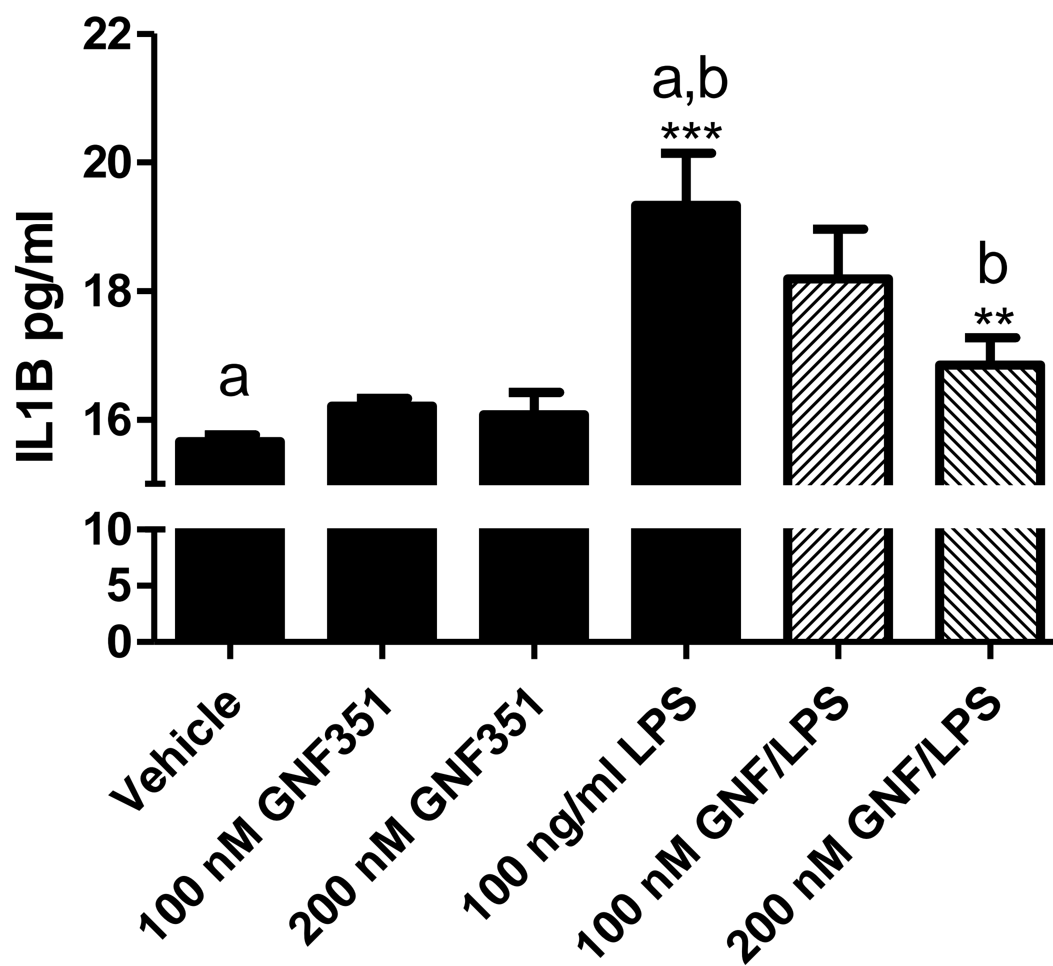

Figure 7. ELISA for secreted IL1B. FLS-RA cells were plated at a concentration of $2x10^6$ cells per well. Cells were first treated for 1 hour with 100 nM or 200 nM GNF351. Cells were then challenged with 100 ng/mL LPS every 12 hours for a total of 48 hours before cell supernatant was collected and analyzed.

ELISA:

Protein levels were assessed using ELISA. FLS-RA cells were stimulated with 100 ng/mL lipopolysaccharide for 4 hours. IL1B was not able to be used to stimulate IL1B production because any added IL1B would have skewed the results as ELISA measures protein concentrations. As expected, addition of 100 ng/mL LPS significantly increased the levels of IL1B in cell supernatant. Treatment with 100 nM GNF351for 1 hour did not significantly reduce IL1B concentration in cell supernatant. However, treatment with 200 nM GNF351 reduced IL1B levels relative to LPS treatment alone. IL1B levels were near the levels observed with controls.

qPCR showed that IL1B stimulation caused increased expression of mRNA, and that GNF 351 attenuates expression. ELISA analysis shows that stimulation with IL1B causes increased release of IL1B to the extracellular environment. While increased expression of IL1B can have significant consequences in the cell through autocrine signaling, increased release of IL1B is perhaps more significant to RA pathology. Increased release of cytokines causes paracrine signaling to surrounding synoviocytes and immune cells, leading to amplification of the positive feedback pro-inflammatory signaling.

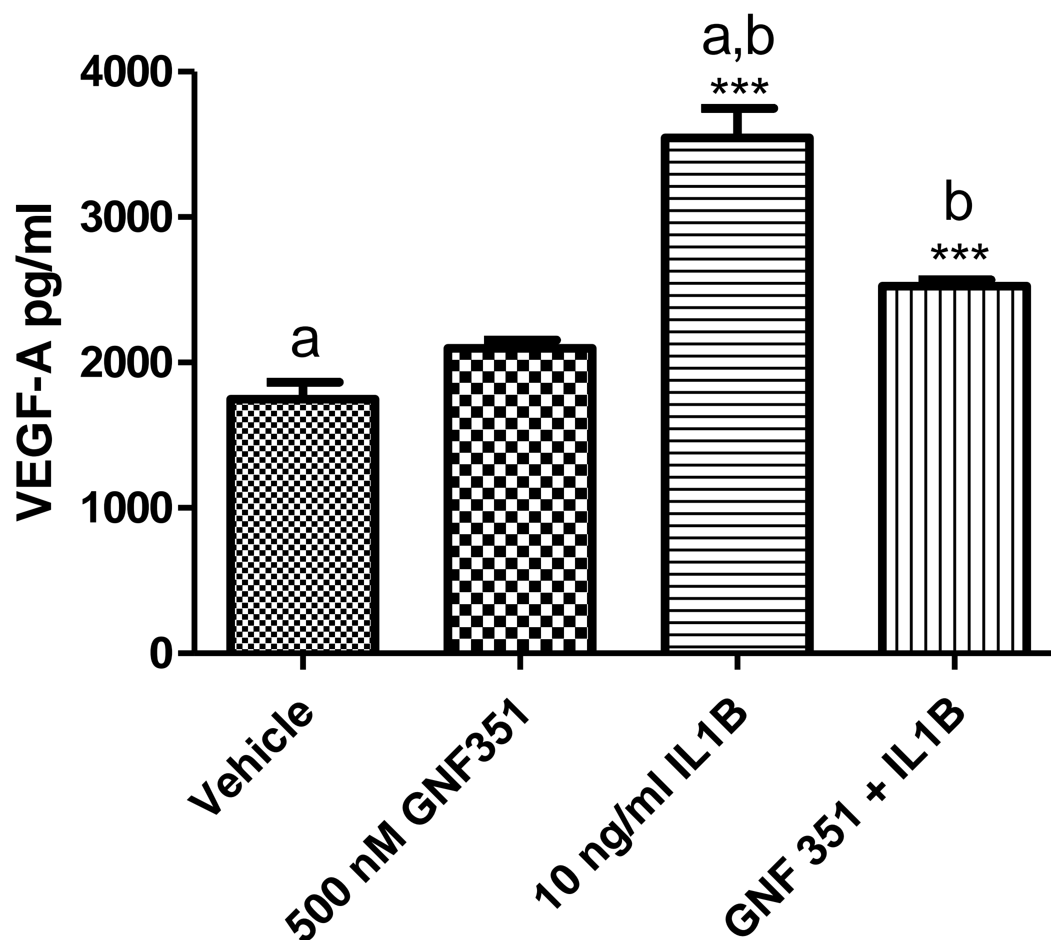

Figure 8. ELISA for secreted IL1B. FLS-RA cells were plated at a concentration of 2x106 cells per well. Cells were first treated for 1 hour with 100 nM or 200 nM GNF351. Cells were then challenged with 10 ng/mL IL1B every 12 hours for a total of 36 hours before cell supernatant was collected and analyzed.

ELISA for VEGFA production in FLS-RA cells showed similar results as for IL1B. Stimulation with 10 ng/mL IL1B caused secretion of VEGFA to almost double relative to vehicle control. For this experiment, AHR antagonism was carried out using 500 nM GNF351. This is a much greater dose compared to previous experiments, but as was shown in the LDH cytotoxicity assay, does not appear to cause toxic effects to the cells at this concentration. GNF351 did attenuate VEGFA secretion to a significant extent.

Secretion of growth factors like VEGFA are part of the reason hyperplastic growth in the pannus occurs, and contributes to invasive properties of the disease. Significantly reducing VEGFA secretion by FLS in vivo could result in more favorable disease manageability and prognosis by confining effected areas.

Conclusions

The AHR mediates expression of proinflammatory genes IL1B and COX-2 in the presence or absence of an inflammatory signal in synoviocytes. This expression is synergistically upregulated with IL1B co-treatment, a prevalent cytokine in the synovium of RA patients. Stimulation of proinflammatory gene expression results in a positive feedback signaling loop amplifying the levels of cytokines both locally and systemically. Therefore, attenuating the production of these molecules is a key to halting the progression of RA.

GNF351 is an AHR antagonist that successfully attenuated IL1B, COX-2, and VEGF-A expression in HFLS-RA cells following stimulation with IL1B. Antagonism of the AHR was also able to attenuate expression of growth factor VEGF-A, which is important in angiogenesis and the invasiveness of RA. Action of GNF-351 was confirmed to be AHR-mediated through siRNA directed AHR gene ablation and qRT-PCR analysis. By ablating AHR, GNF-351 was no longer able to repress IL1B induced gene expression of IL1B and COX2. AHR gene ablation also revealed that the AHR may have constitutive activity in FLS-RA cells independent of exogenous ligand or pro-inflammatory stimulation. This constitutive activity and subsequent gene expression was attenuated through AHR ablation, but not through antagonism with GNF-351. This suggests that the AHR is driving gene expression in FLS-RA cells through an unkown mechanism that may be predisposing cells to a pro-inflammatory character even prior to RA disease onset.

LDH assay in primary FLS-RA cells showed no signs of toxicity upon treatment with GNF-351 at concentrations up to 1 μM. All of the aforementioned results are desirable for a drug to be able to effectively treat RA. By reducing production of pro-inflammatory molecules such as IL1B and COX2, as well as mitogens like VEGFA, AHR antagonism has potential to alleviate severe symptoms of RA and prevent disease progression. AHR antagonism represents a possible novel therapeutic approach in the treatment of RA.

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ACADEMIC VITA

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Education:

2010-Present: B.S. candidate, Toxicology with Honors in Veterinary and Biomedical Sciences and minoring in Biology, College of Agricultural Sciences, Schreyer Honors College, and Eberly College of Science, The Pennsylvania State University, University Park, PA (expected May 2014).

2007-2010: High school diploma, Smethport Area Junior Senior High School, Smethport, PA. Salutitorian.

Experience:

Undergraduate Research Assistant **(Jan 2011-Present):** Pennsylvania State University

- Carry out experiments on Aryl Hydrocarbon Receptor antagonism as related to rheumatoid arthritis, gout, as well various cancer types
- Perform real-time PCR, reverse transcriptase PCR, isolate cellular components such as nucleic acids or proteins, western blots, cell culture, protein assays, cellular transfections, reporter gene assays, and carrying out experiments in mouse models
- Assist in performing Enzyme-linked immunosorbent assays, and chromatin immuno-precipitation
- Publications:
    1. The Ah Receptor Regulates Growth Factor Expression in Head and Neck Squamous Cell Carcinoma Cell Lines (John K , Lahoti TS, Hughes JM et al,) Molec. Carcinog. 2013, April 27.
    2. Aryl hydrocarbon receptor antagonism mitigates cytokine-mediated inflammatory signaling in primary human fibroblast-like synoviocytes (Lahoti TS, John K, Hughes JM et al,) Ann. Rheum. Dis. 2013, October 1 1;72 (10).
    3. Aryl hydrocarbon receptor antagonism attenuates growth factor expression, proliferation, and migration in fibroblast-like synoviocytes from patients with rheumatoid arthritis (Lahoti TS, Hughes JM, Kusdnadi A et al,) J. Pharmacol. Exp. Ther. 2014, February. 348(2) 236-45.
- Poster Presentation: The aryl hydrocarbon receptor regulates the expression of multiple growth factors in highly metastatic head and neck squamous cell

carcinoma cell lines. (John K, Hughes JM,et al,) Presented at 51st Society of Toxicology Meeting, March 10-14, San Antonio, TX.

Skills:

- Strong background in biology, chemistry, biochemistry, and toxicology
- Experience in biochemical research setting
- Proficient in the use of Microsoft Office, GraphPad Prism, and Minitab statistical software
- Basic knowledge of American Sign Language

Activities/Awards:

- Dean's List **(7 semesters)**
- State Farm Companies Foundation Scholarship **(2010-Present)**
- Scholarship for Excellence in Agriculture **(2010-Present)**
- Academic Excellence Scholarship **(2010-Present)**
- Penn State Blue and White Society **(Aug 2011-Present)**
- Representative of the Schreyer Honors College to the University Park Undergraduate Association
  (Apr 2011- Apr 2012)
- University Park Undergraduate Association Academic Affairs Committee representative **(Apr 2011- Apr 2012)**
- Schreyer Honors College Student Council Executive Board **(Apr 2011-Apr 2012)**
- Schreyer Honors College Student Council THON **(Apr 2011- May 2012)**
- Atlas THON **(Aug 2010- Apr 2011)**
- President of Atlas Hall Residents' Society **(Aug 2011- Aug 2012)**
- Schreyer Honors Orientation Mentor **(Aug 2011)**