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Understanding an Inflammatory Pathway in Diabetic Retinopathy

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UNDERSTANDING AN INFLAMMATORY PATHWAY IN DIABETIC RETINOPATHY

A Thesis

by

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ABSTRACT

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Diabetic Retinopathy (DR) is the leading cause of blindness in the U.S. However, not much is known of its molecular pathway and how it attributes to increases in inflammatory response in the eye. One avenue we will investigate is the transforming growth factor beta (TGFB) signaling pathway and its effect of vascular endothelial growth factor (VEGF) secretion and cell viability. VEGF is the hallmark that exacerbates DR progression in prolonged diabetes. Some major concern that have arisen are the underlying effects of oxidants and antioxidants in elevating VEGF secretion in diabetes. In attempt to learn more, we evaluated how an oxidant (acrolein) and antioxidant (hypoxia) impact 661W cone photoreceptor cells in the retina. 661W cells were cultured in DMEM, 10% FBS, 1% AB and once confluent seeded into 6 wells with 300, 000 cells per well. Cells were conditioned in 5.5 and 30mM glucose, in addition to their appropriate treatments of hypoxia induce using cobalt chloride (CoCl2) and various concentrations of acrolein including 25, 50, 100, 200uM for a 24-hr. treatment period. Following the collection of conditioned media to measure VEGF secretion and cell viability to quantify number of viable cells using the hemocytometer. Moreover, to determine the role of TGFB signaling pathway inhibition will block the molecular pathway to determine how VEGF secretion and cell viability are affected in the respective treatments listed above. Based on the

data collected hypoxia has a significant impact on increasing the amount of VEGF secretion p=.002 and decrease cell viability p=.028. Additionally, acrolein played a significant role in decreasing cell viability and VEGF secretion in a dose dependent manner in 661W photoreceptor cells. Due to hypoxia and acrolein being known to affect oxidative pathways significantly, it is possible that their effects may be mediated by the TGFB pathway. Moreover, it is suggested that there is an additional increase in VEGF secretion and decrease in viable cells after inhibition of TGFB allowing us to believe that there is an additional part of the pathway that is contributing to these effects. Overall, hypoxia exerted a significant effect to reduce 661W cell viability and increase VEGF secretion and acrolein caused reduction of cell viability along with a decrease of VEGF secretion. Acrolein decreased the amount of both VEGF and cell viability in a dose dependent manner. To determine the role of TGFB signaling pathway, two inhibitors were used SMAD/SIS (1) and TGFB receptor 1 kinase (2) to inhibit the pathway from activation by inhibiting the receptor and inhibiting phosphorylation from occurring. By doing this we discovered that inhibitor 2 reduced the hypoxic induced VEGF increase in both NG and HG suggesting pathway involvement. Furthermore, we discovered that inhibitor 2, only, resulted in an increase of viable cells suggesting possible involvement as well. All in all, it seemed that the inhibitor 2 was effective in decreasing the VEGF secretion and increase viable cells to alleviate or reverse the effects seen in DR that include increased VEGF and decrease in viable cells.

DEDICATION

The completion of my studies would not have been possible without the love and support of my family. My mother, Maria H. Castillo, my father, Rene Rodriguez, my sisters, Rina, Renee, Reneal, my brother, Andy, my niece, Sarah, my nephews, Luke, Vince, and Justin and my doggo Princess Rae Rodriguez. Thank you for your patience, love, and support.

ACKNOWLEDGMENT

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I would also like to thank my lab members for working with me through the hard times to get started with research, from shadowing, training, experimental trials, data analysis, and data collections; we made this happen and I will forever remember that.

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CHAPTER I

INTRODUCTION

Diabetes

Diabetes mellitus is one of the most common chronic-degenerative diseases in adult populations; type 1 is characterized by the inability to produce the typical amounts of insulin, and type 2 diabetes is identified by the body's reduced sensitivity to insulin [1]. Diabetes is affected by the oral intake of high glucose foods, which is found in a variety of commonly consumed foods. Due to these prolonged hyperglycemic conditions found in diabetes, there is evidence to suggest significantly increased levels of various inflammatory responses and significant decreases in antioxidants that are protective of diabetic conditions. Prevalence of diabetes in the world was 6.4% of the population, however, researchers estimate that the prevalence of diabetes will increase to 7.7% by 2030. [2]. Diabetes, a growing disease not only at the global level, but more specifically in developing counties and in the Rio Grande Valley (RGV) [2,3]. The significant increases of inflammatory responses seen show importance in investigating diabetic conditions that have potential to further prevent or treat from exacerbated effects. Patients with diabetes have shown to have upregulation of oxidative stress, cytokines, and proteins that are significantly altered by the hyperglycemic conditions present on the body, specifically the eye [1]. Diabetic diseases that occur in the eye include diabetic retinopathy (DR), diabetic macula edema (DME), cataracts, glaucoma [4]. Over time, these diseases begin to

damage the eye, leading to vision loss and ultimately blindness. Here we will focus on diving into the molecular pathway associated with understanding the pathological diseases of Dr.

CHAPTER II

REVIEW OF LITERATURE

Diabetic retinopathy

DR is the leading cause of blindness in the U.S. [5]. DR is affected by the upregulation of glucose over a long period of time in diabetic patients. Due to the rising prevalence of diabetes that can be either inherited or acquired over time, from unhealthy eating choices and other factors that put you at high risk it is important that we look at other therapeutic intervention or treatments to decrease the progression of the diseases or treat it. DR is a severe complication in which the blood vessels in the back of eye (the retina) begin to leak causing angiogenesis; this process occurs when newly formed blood vessels proliferate causing loss of vision, ultimately leading to blindness. Additionally, lab and clinical evidence suggests that microvascular, inflammation, and retinal degeneration may significantly play a role in the progression of diabetic retinal damage seen at early stages of DR [1]. Currently, there are treatments available to help reduce the progression of the diseases however, still little is known of DR. Treatments currently available include laser photocoagulation, vitreous, surgery, intravitreal agent, and anti-VEGF agents that serve as a therapy for DR [6].

DR has a plethora of pathways associated with hyperglycemia induced retinal damage that including oxidative stress [7], advanced glycation end products (AGEs) accumulation [8], TGFB [9], inflammation responses [10], and VEGF [11]. VEGF in proliferative diabetic retinopathy (PDR) in previous studies is the hallmark of this disease. Glucose induced levels of

VEGF have been associated with being an inflammatory cytokine found DR in multiple cell lines. VEGF has been shown to increase angiogenesis and proliferation of blood vessels seen in DR. Previous studies, have shown significant increase in oxidative stress conditions including increased levels of VEGF secretion in cone photoreceptors, something that was not very commonly known to occur which calls for a deeper investigation of the retinal damage being affect in hyperglycemic found in DR. Rods and Cone photoreceptors play a vital role in the usage of oxygen in the retina, hence once rod cells are damaged, the oxygen levels being exposed to cones begin to cause oxidative damage to their cells leading to cell death and increasingly alarming levels of other cytokines and proteins. Different retinal pathways are currently under investigation to determine how we can target specific markers to inhibit the exacerbated effects that cause vision loss in the retinal diseases.

Oxidative stress seen in Diabetic Retinopathy

In oxidative conditions the protein structures are changed, playing a significant role in the pathogenesis of diabetic retinopathy. Previous studies show that in low levels of oxidative stress proliferation, inflammation, and apoptotic conditions are induced. In contrast, in high levels oxidative stress will lead to detrimental effects such as mutations found in DNA. Moreover, studies have shown that hyperglycemia can cause increasing oxidative stress in animal models. Significantly showing increased levels of VEGF secretion in hyperglycemic conditions as well as, oxidative stress [7].

Oxidative stress, as seen in DR, has potential to cause inflammation, cell death, apoptosis, and vascular and damage to the retina [7]. Du et al. constructed a diagram that leads us to the schematic view of diabetes, increase reactive species, inflammatory changes, DR. This allows us to discover why there is an increase of the reactive species (oxidative stress) and how

this leads to the possible prevention of inhibiting the pro-inflammatory response from exacerbating the progression of DR [9]. In Kurihara et al., researchers suggested that though photoreceptors may not be directly impacted by the oxidative stress (hypoxia) they can induce the promotion of degeneration of the photoreceptors as seen in retinas of mice exposed to hypoxic conditions [8]. Additionally, it has been confirmed by Wellard et al., that rat retinal photoreceptors do in fact die off, as seen in TUNEL assay, as a result of hypoxic exposure and included that adult SD retina and mouse models are showed similar results [10].

Photoreceptors are responsible for secreting interphotoreceptor retinol binging protein (IRBP), an antioxidant to maintain balance of ROS. However, in hyperglycemic conditions, ROS in increased, leading to IRBP synthesis inhibition, ultimately leading to photoreceptor cell death. Wholesomely, the retina is an important generator of ROS and has been previously shown to be involved to result in retinal diseases [11]. This gives us insight that oxidative stress plays a significant role in the pathogenesis of DR. In low ROS conditions there are altering of protein structures in which changes the function of those proteins; this has been seen in signal transduction pathways that include the function of proliferation, inflammation, apoptotic and gene expression, like TGFB. Moreover, high ROS can cause detrimental effects to the health of DNA leading to mutations, ultimately causing cell death. Animal studies have previously shown that an increase of ROS generation is accumulated in the presence of hyperglycemia conditions [10]. It is noted that the association of increased ROS is linked to mitochondrial DNA damage. Retinal endothelial blood barrier damage caused by ROS leads to the photoreceptor damage. Being that oxidative stress is increased due to underlying diabetic conditions, understanding the molecular pathway of oxidative stress on 661W cone photoreceptors is needed [11, 12, 13, 14].

Acrolein's effect on Diabetic Retinopathy

Acrolein can be found in the environment, in dietary substances, and in the smoke that is secreted through burning fumes in cigarettes in substantial quantities [15,16]. Acrolein is an unsaturated aldehyde, an antioxidant, found in the fumes of greasy foods. Some of the diseases acrolein has been associated with include various ocular diseases such as DR, retinal detachment, and glaucoma [17]. However, not limited to ocular disease acrolein also has been shown to be linked to many other diseases [16]. Some studies suggest oxidative stress damage occurring due to the induction of acrolein however, not much is known on how acrolein affects the synthesis and production of VEGF secretion determining the severity of PDR.

This review involves acrolein as a target to determine its effects in mediating increased oxidative stressors in DR to increase the quantity of cytokines that can be affected. Acrolein has shown to have other contributing factors to DNA damage, inflammation, and ROS formation. It is important to observe these changes in the photoreceptors due to their contribution of antioxidants, such as IRBP, in the retina. Here, the pathway that is of my interest from this article is that acrolein, causes a depletion of anti-oxidants perhaps IRBP as well-maybe, which hence causes oxidative stress; it is not labeled here however, oxidative stress has shown to increase the VEGF secretion in photoreceptor cells. Could this be why? Because the acrolein directly affects the amount of IRBP? Additionally, there has been a link from acrolein to growth factor increase- which goes back to the following pathway listed above if, there is an increase of TGFb will that later along the line causes an additionally induction of VEGF secretion [18].

Acrolein, show to be both an oxidant most commonly is found in everyday environments as well as, in cigarettes in substantial quantities. Previously, studies have shown association with various ocular diseases including diabetic retinopathy. Studies have shown that this occurs

because acrolein leads to elevated levels of oxidative stress investigated by ROS. Acrolein has shown to have DNA damage, inflammation, and ROS formation in past studies which allows us to believe that acrolein can cause a significant amount of damage to photoreceptors which exacerbate the effects of diabetic retinopathy or progression or proliferative diabetic retinopathy.

Vascular endothelial growth factor increased in Diabetic Retinopathy

VEGF has been said to be the hallmark of DR, as it has seen to be significantly elevated in hyperglycemic conditions, as well as in hypoxic in the retina. However, VEGF is also known to play a vital role in the development of new vasculature [11]. Additionally, we also previously showed that there is a very large increase in conditions of hypoxia however, glucose seems to play a small role in terms of DR. Though DR is known to causes retinal hypoxia to drive these pro-angiogenic growth factors to be increase exacerbating the effects of proliferative DR.

Transforming growth factor beta (TGFB) possible role in Diabetic Retinopathy

TGFB superfamily is responsible for many of the cellular processes including proliferation, differentiation, cell death, adhesion, and migration. The TGFB superfamily in mouse models play a detrimental role in the development as well as, mediating antiproliferative effects in epithelial cells, regulation of immune, and healing responses. Many diseases have associated TGFB family signaling to be detrimental to health, and mutations that cause altering of proteins that lead to a series of diseases. SMADS are involved in the signaling cascade of TGFB pathway by directly sending signals from cell surface transmembrane receptors to the nucleus through intracellular mediators. Inhibition of SMADs, induced by TGFB signaling resembling a negative regulator of the pathway by binding to the type 1 receptor, thus competing

with R-SMADS and blocking the phosphorylation from occurring [19]. TGF alpha and TGF beta are both components involved in the pathway leading to diabetic retinopathy [20].

Lee et al. studied the effects TGFB and the notch signaling pathways, in conjunction with SMAD3 pathway in the proliferation of zebrafish. TGFB is said to enhances extracellular matrix production after injury; proliferation of cells is very crucial for the regeneration of these cells. TGFB signaling pathway was of interest, since it has been seen upstream of proliferation. Researchers have tested TGFB1 and TGFB3 in various zebrafish models. Zebrafish were previously tested and had significant release of different growth factors and cytokines after injury, possible stress, and exacerbation of effects that would ultimately lead to cell death. Zebrafish have a high density of cone photoreceptors [23]. Previously studies, have shown that tissue infarction and impaired heat regeneration is possible by chemically inhibiting the TGFB pathway using a chemical inhibitor SB431542. This inhibitor blocks the TGFB type 1 receptor of TGFB. Additionally, results indicate Smad2/3 mediated TGFB signaling acts to inhibit proliferation of neuronal progenitors following photoreceptor destruction in zebrafish retinas. TGFB pathway is unclear, so they inhibit the SB431542 during retinal regeneration after inducing MNUinduced photoreceptor degeneration. Here they also observed retinal regeneration after the inhibition of the TGFB pathway [22]. TGFB was observed to see how TGFB affected the proliferation and regeneration of neuronal retinal glial cells in the retina of zebrafish by Cia in 2020. They associated TGFB signaling pathway with downstream of SMAD pathways, leading to proliferation. This would mean that by inhibiting the TGFB receptor, we would inhibit the pathway of TGFB as well as inhibit the effects caused by SMAD. Since it was said that TGFB could stimulate the expression of SMAD. Ultimately, if we inhibit TGFb1 or 3 there will not be proliferation that is seen after SMAD down this pathway, additionally another mechanism

is to simply inhibit the SMAD which is after the TGFB signaling pathway. All these factors can lead to the damage and destruction of vessels in the retina, photoreceptors included due to them having VEGF and TGFB expression, ultimately leading to enhanced retinal neovascularization as seen in DR [21,22]. VEGF secretion upregulation, which is the hallmark of angiogenesis in the development of proliferative diabetic retinopathy caused by hyperglycemic conditions [24]. The effects of hyperglycemia in the retinal blood flow, basement membrane thickening, and growth factors associated with increase glucose. Questions that continue to arise are the connection between growth factors including VEGF and TGFB. Studies suggested that in the presence of hyperglycemia growth factors increase, significantly inducing the remaining pathway that lead to AGEs and oxidative stress induction, inducing retinal hypoxia, leading to an increase in growth factors specifically, VEGF [23].

With VEGF being an important protein causing the progression of DR to be exacerbated at high quantities cell studies have tried to investigate the secretion and expression of VEGF in human retinal pigment epithelial (HRPE) cells and their involvement with the TGFB signaling pathway. Studies show that in hyperglycemic conditions induced levels of VEGF 121, 165, and 189 presents due to upregulation with TGFB pathway [25]. However, human retinal pericytes studies have been used to test the effects of TFGB1 and TGFB2 in the induction of BIGH3 protein and apoptosis, here they also tested for the role of increasing concentration of TGFB and tested for BIGH3 protein. This led us to conclude that the increase of the BIGH3 causes loss of retinal pericytes during early events in diabetic retinopathy [26]. This is an important finding since other cells can potentially have a degree of inflammatory response changing the amount of VEGF secretion secretes via photoreceptors. Moreover, these studies, suggested that TGFB pathway had significant impact on the VEGF secretion by HRPE cells. Being that degeneration

and loss of pericyte and RPE cells lead damaged capillaries this ultimately leads to damage to the retinal photoreceptors in which partial or complete blindness may occur.

CHAPTER III

METHODOLOGY

Cell culture: Cone photoreceptor (661W) cells and maintained at 37 degrees Celsius in 5% Carbon dioxide and 95% oxygen incubation. Cell culture media consists of 5% fetal bovine serum, 1% penicillin streptomycin, and DMEM medium. During the culturing processes PBS (1x) will be used for cleaning and Trypsin for the detachment of cells from p100 dishes. In centrifuge, optimal range used is 300gs, for 3mins, at 14 degrees Celsius to seed and culture. Cells will be cultured in 6 wells with 300,000 cells per well.

Treatments: The treatments that will be tested for this study: 5.5mM glucose, 30mM glucose, 300x hypoxia, and acrolein concentrations of (0, 25, 50, 100nm). Glucose Treatments: The concentrations used will be Normal 5.5mM glucose and Hyperglycemic 30mM to represent the diabetic retinal cells. Hypoxia: To represent the hypoxic state, I will be using $CoCl_2$ to induce the low oxygen level environment. Concentration used will be at 300x for 24 and 48hrs.

Acrolein Treatments: The concentrations that will be used will be dilutions to (0, 25, 50, 100ng/mL)

TGFB pathway inhibition: Cells were pretreated with SB431542 in DMSO (sigma no. S4317) and TGFB1 receptor kinase

Mouse VEGF ELISA: To determine the VEGF secretion the use of mouse VEGF ELISA kit will be used (catalog number: MV000). After 24-hour treatment, 1mL of the condition media

collection from the treated plates will be stored at -80 or quickly analyzed through VEGF ELISA. In this study we will be using (R&D system, MV100 Mouse VEGF Elisa Kit). Enzyme-linked immunosorbent assay is a lengthy procedure taking about 6-7 hours. The data produced by the ELISA consists of a standard curve and concentrations of VEGF per sample (pg./mL). This is done by preparing a standard using anti-VEGF along with other diluents, then analyzing the protein product using antibodies that link to specific indicators that are present using absorbance.

Cell count:With the remaining cells in the dish, I performed cell counts using a Hemocytometer with Trypan blue staining to calculate the number of viable cells after treatment exposure.

Statistics: Data was analyzed to get a mean calculation for each sample in order allow for accurate reproducible data. An ANOVA to determine the differences between one or more group means from each trial will be done. If the F ratio is equal or close to 1 the variance between the groups is not true. Additionally, the one way will help to determine what influence an independent variable (treatment) has on the dependent variable (VEGF secretion) in this study.10

CHAPTER IV

RESULTS

For preliminary data studies, looking at how 661W cell viability is affected in different concentrations of glucose and acrolein was something of interest that way we can determine they affects these conditions have. According to figure 1, the MTT results showed significant increase of cell viability between the lower concentrations 5.5mM of glucose and 30mM. In figure 2, MTT results showed that between 0 and 5uM acrolein dose there was a significant amount of viability however, at 50, 100, and 200uM the cell viability was significantly decreased. Moving forward, since the use of hypoxia was used, figure 3 shows the significant decrease of oxygen presented in the hypoxic treated dishes in the presence of cells exposed to the hypoxia induced treatment $CoCl_2$ at 300uM.

Aim 1, was to determine how glucose, hypoxia, and acrolein affect VEGF secretion and cell viability hand in hand in different treatment concentrations of the respective treatment either the antioxidant (hypoxia) or oxidant (acrolein). In figure 4-6, VEGF secretion showed significant increase between normal and hypoxic groups in both low and high glucose. Additionally, viable cells exposed to hypoxic conditions showed significant decrease. Looking at both, VEGF and cell viability in 661W it seems that in the hypoxic conditions there is significant results that show increasing levels of VEGF secretion per cell in these conditions. Observe again VEGF and cell viability, in acrolein exposure to 661W cells the concentration of VEGF secretion was significant decreased as the dose of acrolein increased. Drastic decreases of cell viability were seen in the

acrolein dose dependent. These results showed to be no significance between the concentrations of acrolein.

Aim 2, was approaching different sections in which photoreceptors may be affected as a result of prolonged hyperglycemia seen in diabetes however, the avenue of interest here is of hypoxia and toxin exposure and how this affects the severity of DR as represented by the VEGF secretion of 661W photoreceptors. Results only concluded that in the TGFB receptor kinase inhibitor for both VEGF and viable cells there was a reverse effect occurring decreasing VEGF secretion and increasing viable cells shown in figures 13 and 19.

CHAPTER V

CONCLUSION

Retinal hypoxia plays a significant role in increasing VEGF secretion and decreasing viable cells. However, inhibition results suggest an association of TGFB pathway with hypoxia-induced VEGF increase and reduction of viable cells. Acrolein-induced decrease in VEGF and reduction of viable cells are not associated with the TGFB pathway. Moreover, there was no clear distinction between normal and high glucose conditions. Treatment therapies can be focused on alleviating the effects of exacerbating PDR and decrease the pro-angiogenic growth factor affects.

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APPENDIX

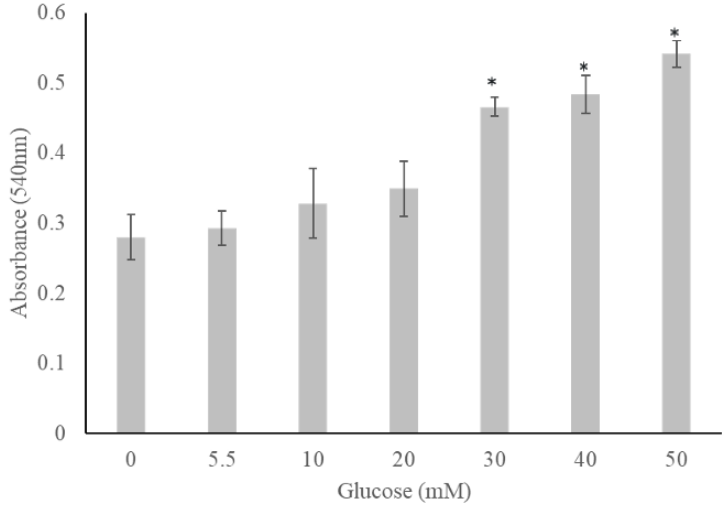

Figure 1. 661W Cell viability in various glucose concentrations including 5.5, 10, 20, 30, 40, 50mM in complete media after 24-hr.

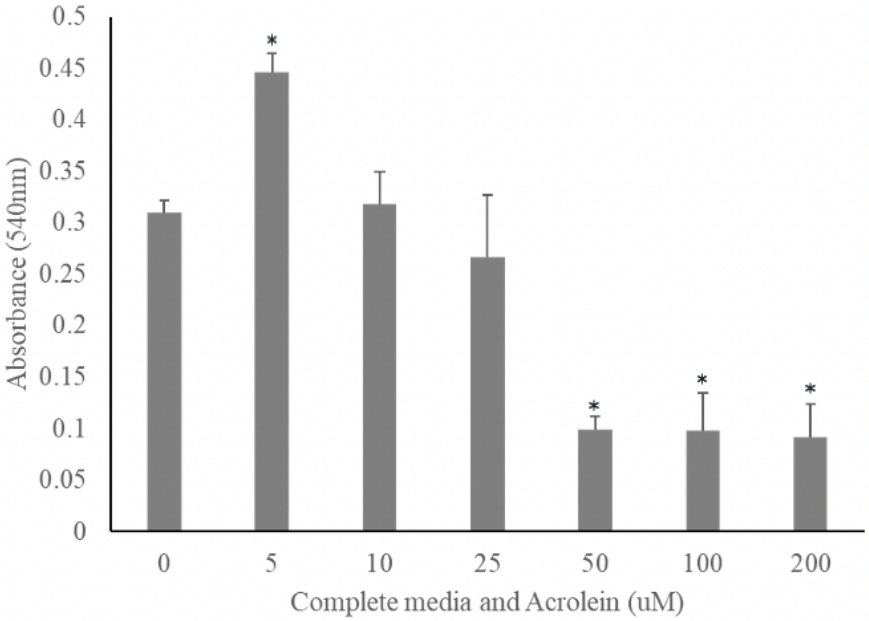

Figure 2. 661W Cell viability observed in different acrolein doses including 25, 50, 100, 200uM in complete media after 24-hr.

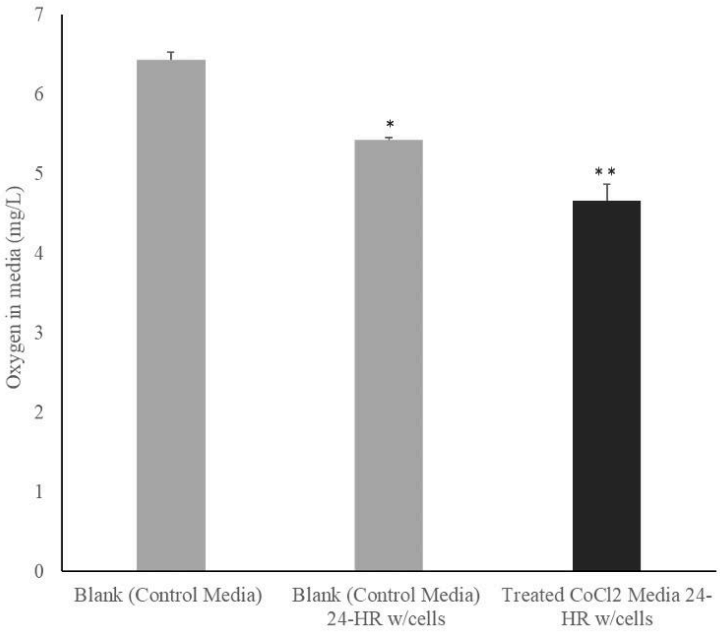

Figure 3. Dissolved oxygen (mg/L) in conditioned media before and after 24-HR CoCl2 treatment with/ without 661W cells.

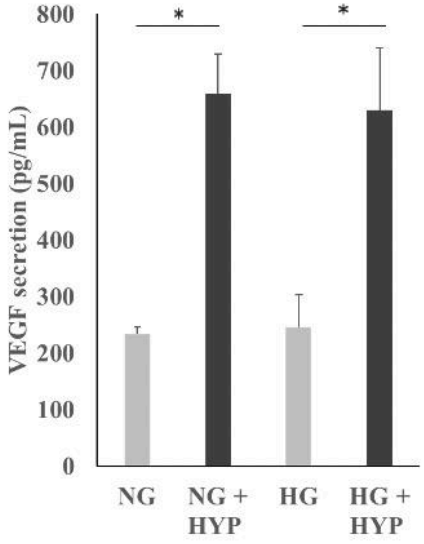

Figure 4. VEGF secretion in 661W in normal and hypoxic (HYP) conditions for 24 hours incubation period in both 5.5 mM normal glucose (NG) and 30mM high glucose (HG).

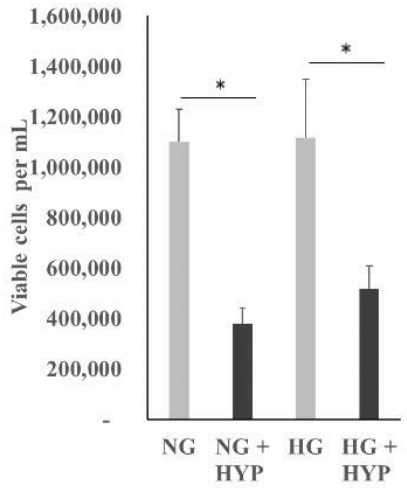

Figure 5. Viable 661W cells in normal and hypoxic conditions for 24 hours incubation period in both (NG) 5.5mM and (HG) 30mM.

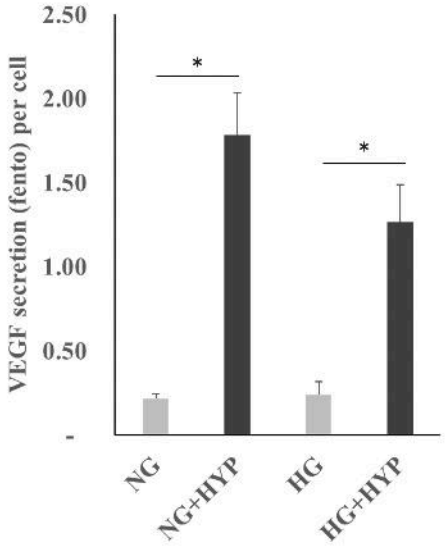

Figure 6. VEGF secretion per cell in 661W cells in normal and hypoxic conditions for 24 hours incubation period in both (NG) 5.5mM and (HG) 30mM.

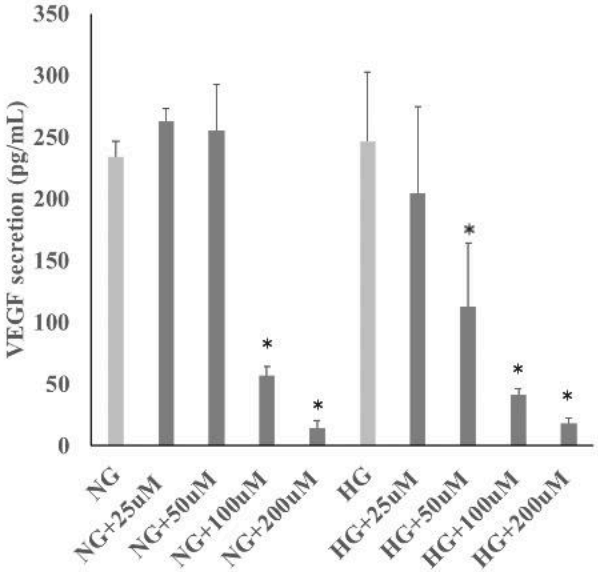

Figure 7. VEGF secretion in 661W cells in NG and HG with 24-hour incubation treatment period with Acrolein concentrations included are 25, 50, 100, 200uM.

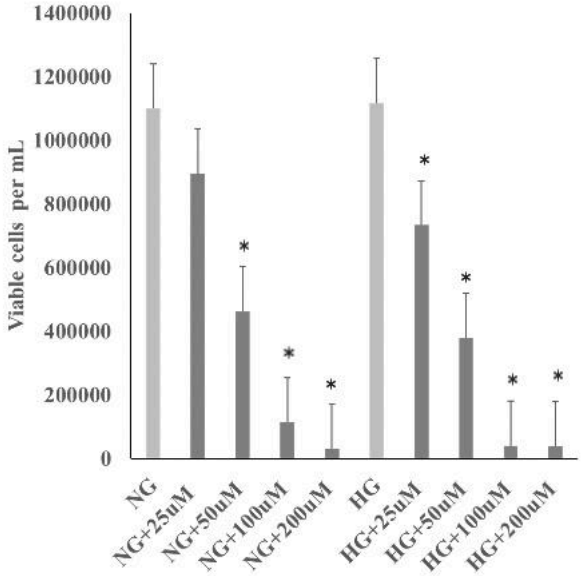

Figure 8. Viable 661W cells in NG and HG with 24-hour incubation treatment period with Acrolein concentrations included are 25, 50, 100, 200uM.

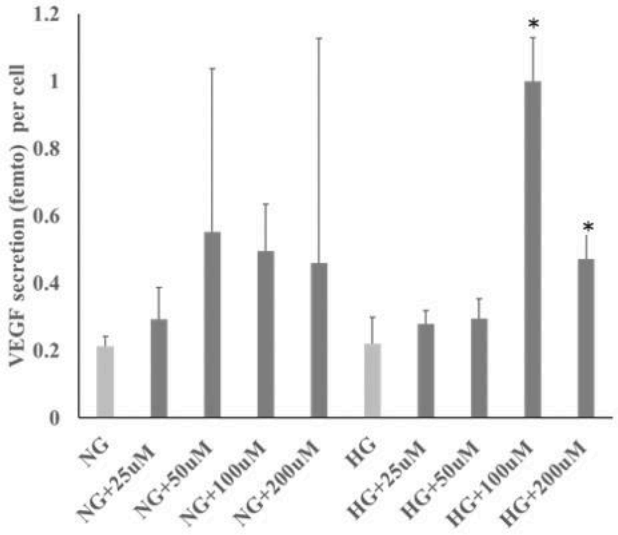

Figure 9. VEGF secretion per cell in 661W cells in NG and HG with 24-hour incubation treatment period with Acrolein concentrations included are 25, 50, 100, 200uM.

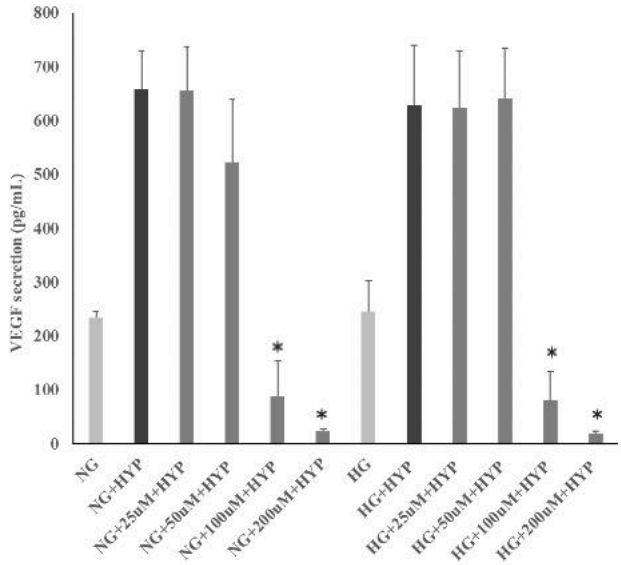

Figure 10. VEGF secretion of 661W Cone Photoreceptor cells in hypoxic conditions with the presence of acrolein concentrations including (25, 50, 100, 200uM) for 24-hour treatment.

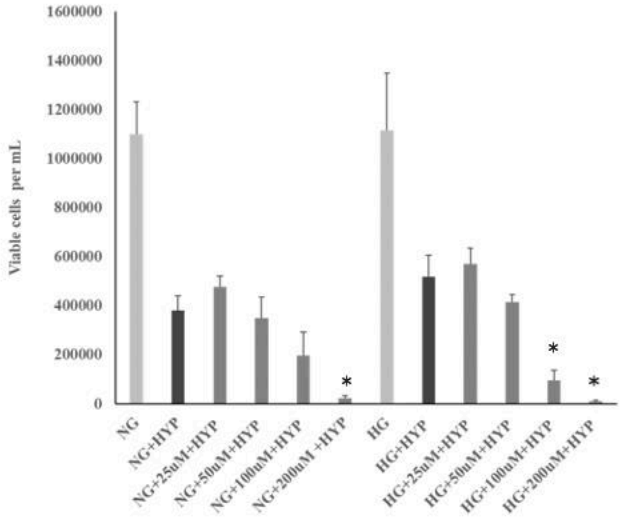

Figure 11. Viable 661W Cone Photoreceptor cells in hypoxic conditions with the presence of acrolein concentrations including (25, 50, 100, 200uM) for 24-hour treatment.

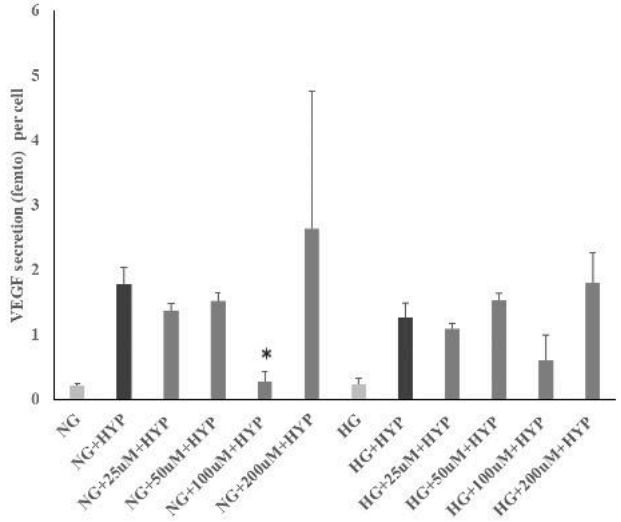

Figure 12. VEGF per viable 661W Cone Photoreceptor cell in hypoxic conditions with the presence of acrolein concentrations including (25, 50, 100, 200uM) for 24-hour treatment.

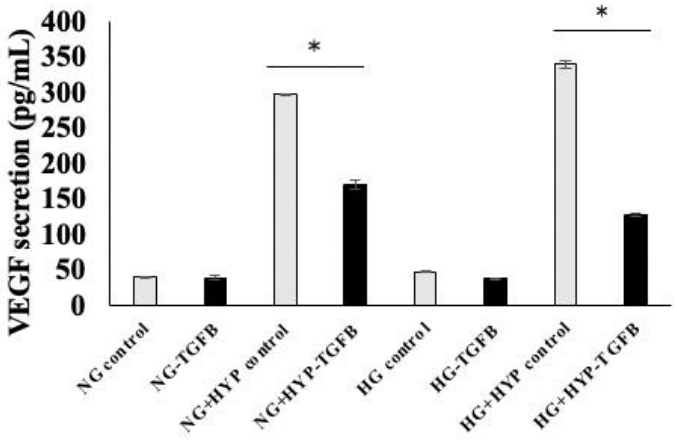

Figure 13. VEGF secretion in 661W cells in hypoxic conditions after inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

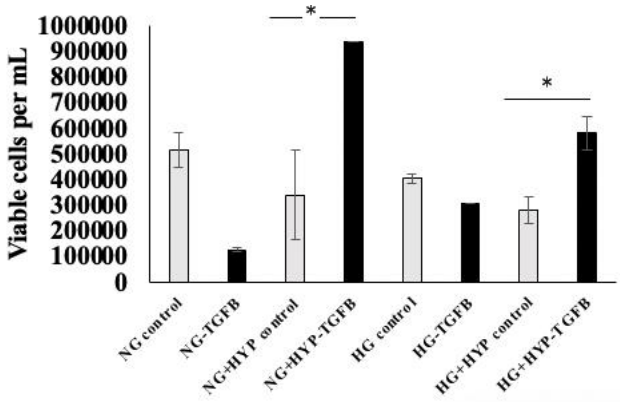

Figure 14. Viable 661W cells in hypoxic condi Horizontal (C inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

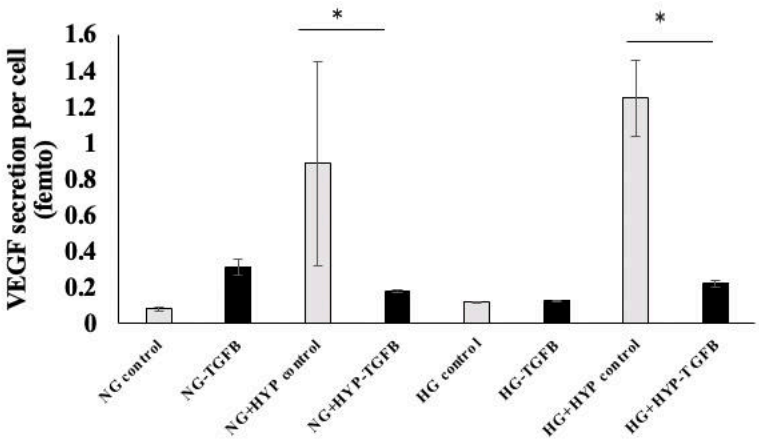

Figure 15. VEGF secretion per 661W cells in hypoxic conditions after inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

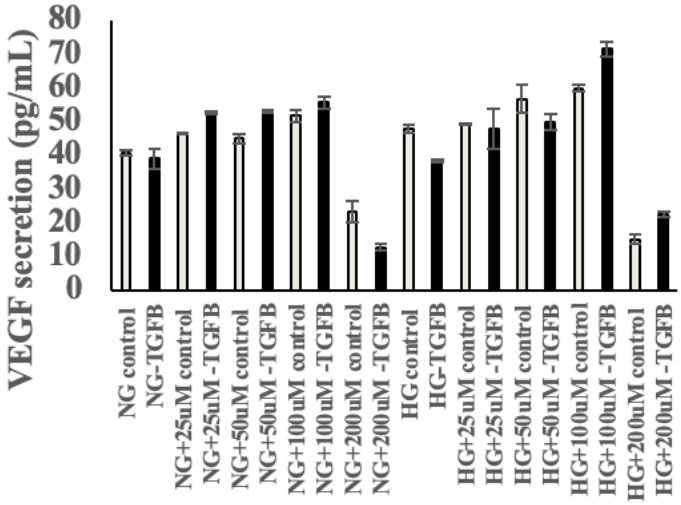

Figure 16. VEGF secretion in 661W cells in various acrolein conditions after inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

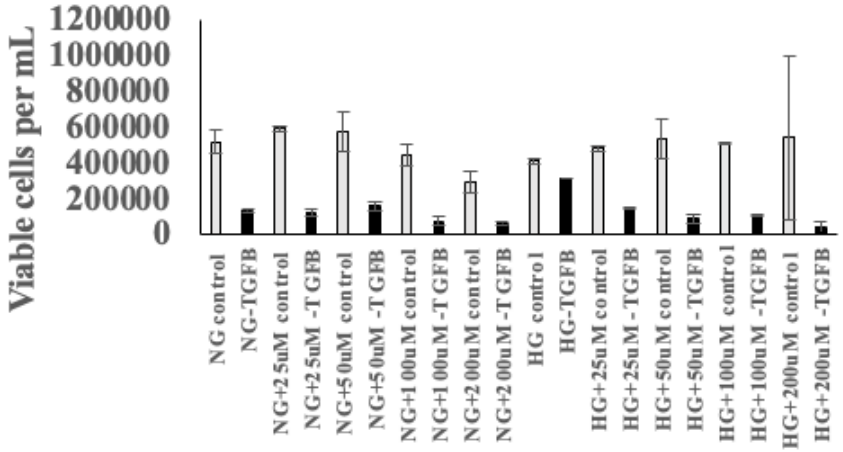

Figure 17. viable 661W cells in various acrolein concentrations after inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

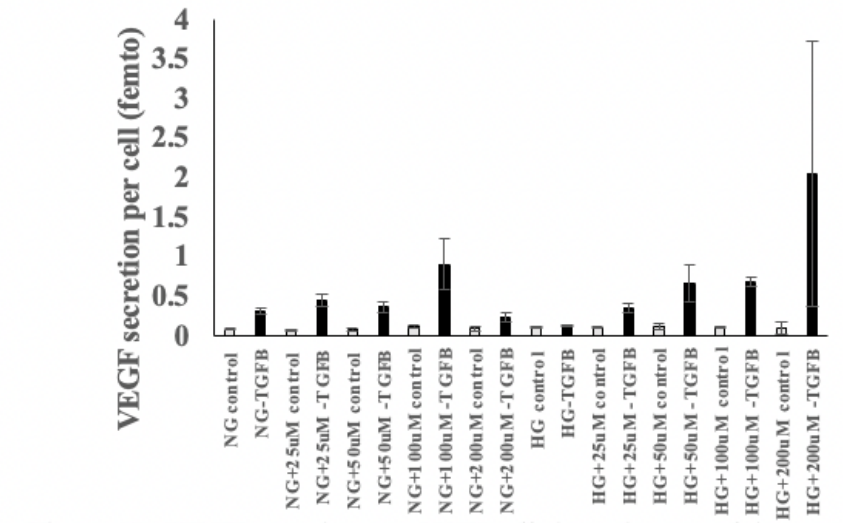

Figure 18. VEGF secretion per 661W cells in various acrolein concentrations after inhibition of the TGFB receptor kinase 1 inhibitor. (NG control- DMSO baseline control)

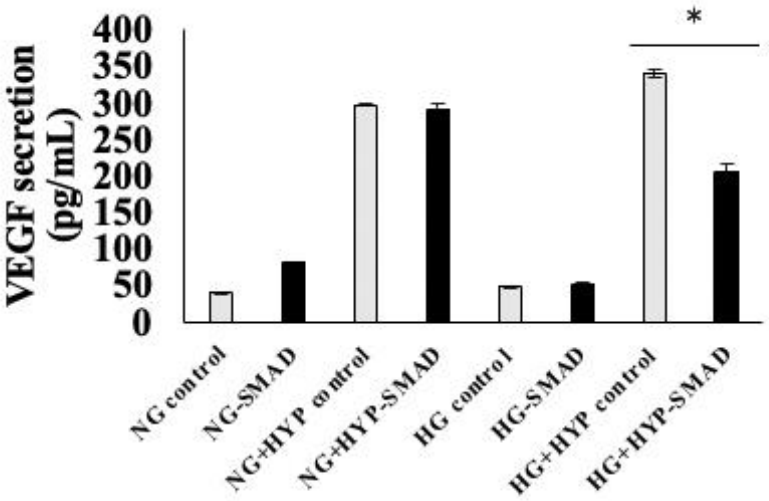

Figure 19. VEGF secretion in 661W cells in hypoxic conditions after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

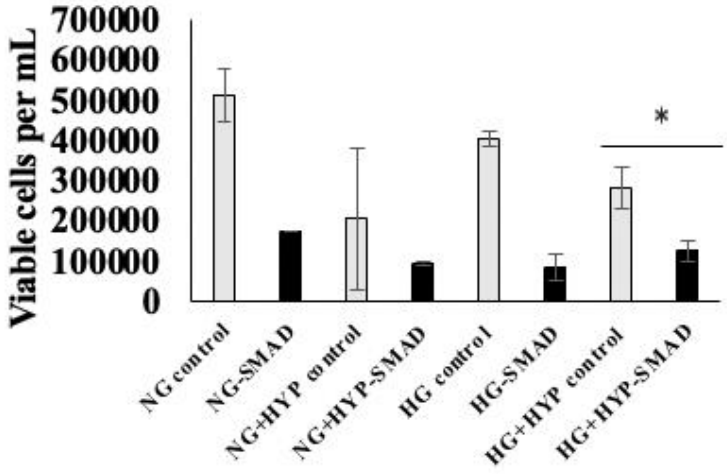

Figure 20. viable 661W cells in hypoxic conditions after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

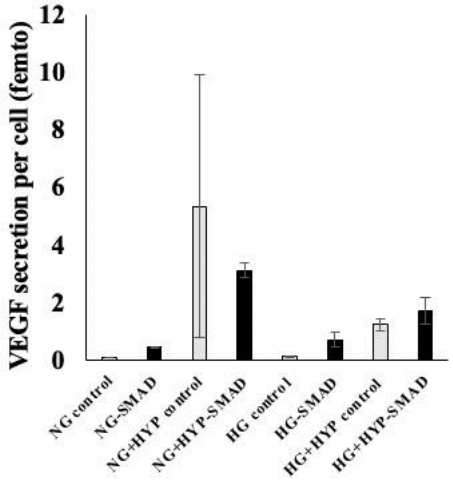

Figure 21. VEGF secretion per 661W cells in hypoxic conditions after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

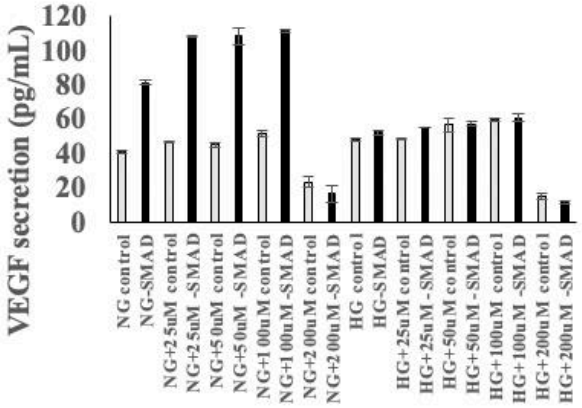

Figure 22. VEGF secretion in 661W cells in various acrolein concentrations after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

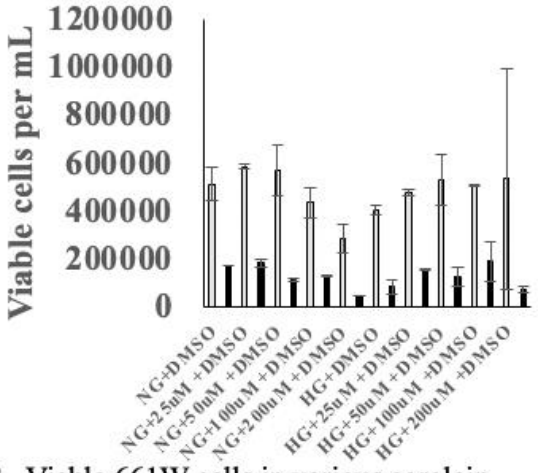

Figure 23. Viable 661W cells in various acrolein concentrations after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

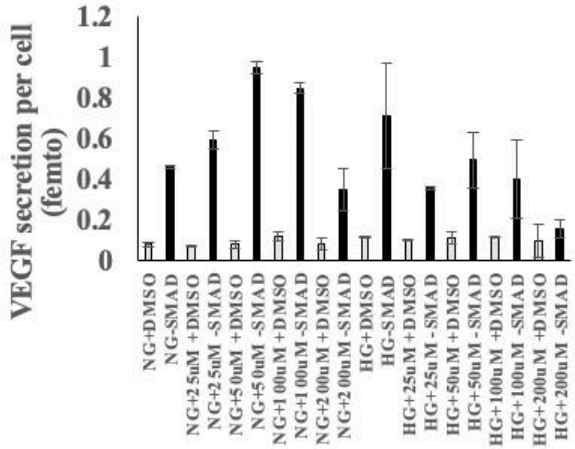

Figure 24. VEGF secretion per 661W cells in various acrolein concentrations after inhibition of SMAD/ SIS inhibitor. (NG control- DMSO baseline control)

BIOGRAPHICAL SKETCH

The author is, Reanna Raye Rodriguez from Edcouch, Texas. Reanna started her academic career at Edcouch- Elsa High school graduating in 2014. Pursued her Biology Bachelor's degree University of Texas Pan American (UTPA), where it quickly transitioned into University of Texas at Rio Grande Valley (UTRGV) from 2014-2019. With passion and ambition, Reanna continued her academic education doing research at UTRGV and was awarded her Master of Science in Biology May of 2021.

Reanna has published abstracts and manuscripts including: Effects of Glucose and Hypoxia on VEGF Secretion in 661W Cone Photoreceptors: Understanding the Mechanism of Diabetic Retinopathy; Acrolein and Hypoxia Induced VEGF Secretion by 661W Cone Photoreceptors; Reduced Expression of Interphotoreceptor-Retinoid Binding Protein (IRBP) in Diabetic Retinopathy – Cell and Postmortem Study; and Diabetic Retinopathy: Targeting BIGH3 to Develop Novel Molecular Therapies. During the SOM 2021 Colloquium Reanna was selected by the Rio Grande Valley- SFN Chapter as Best Neuroscience Poster.

With great aspiration to grow in the field of biomedical sciences, Reanna has continuously done various volunteer work in the healthcare setting and will be applying for the 2022 medical school cohort.

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