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2016 World Congress on In Vitro Biology, June 11-15, San Diego, California

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ANIMAL INTERACTIVE POSTER SESSION ABSTRACT

IN VITRO ANIMAL CELL SCIENCES INTER-ACTIVE POSTER SESSION—Tuesday, June 14, 1:30 pm–2:30 pm

A-3010

The NMP1 Inhibitor NSC348884 Affects Oligomerization and Induces Cell Death in Neuroblastoma Cells. KELLY R. VLCEK[1], Ryan Kelsch[2], Nil Akgul[1], and Kolbrun Kristjansdottir[1]. [1]Midwestern University, Biomedical Sciences Department, College of Health Sciences, Downers Grove, IL and [2]Midwestern University, Chicago College of Osteopathic Medicine, Downers Grove, IL. Email: kvlcek@midwestern.edu

Nucleophosmin-1 (NPM1) is a nucleolar protein involved in several cellular processes including ribosome biosynthesis, cell proliferation, apoptosis, molecular chaperoning, and centromere duplication. Dysregulation of NPM1 has been observed in several cancers including prostate, colon, and breast cancer. Recently, our lab has detected increased levels of NPM1 in neuroblastoma, a solid tumor often found in the adrenal glands of young children. Changes in NPM1 levels can correlate with tumor progression, therefore it is a potential target for cancer treatment. NSC348884, a small molecule inhibitor of NPM1, has been shown to disrupt oligomerization of NPM1 in breast, prostate, and lymphoma, resulting in inhibition of function and increasing cellular apoptosis. The focus of our study is to test the effects of NSC348884 on neuroblastoma cancer cells which have yet to be investigated. Here, we report a concentration-dependent decrease in metabolic activity of cells in SY5Y and WS neuroblastoma cell lines treated with 1–10 μM NSC348884 for 24 h as measured by WST-1 assay. The use of Annexin V flow cytometry assays examines the levels of apoptosis resulting from treatment with the inhibitor. In addition, the oligomerization status of NPM1 in neuroblastoma cells was investigated through the application of Native PAGE following treatment with NSC348884. Understanding the role of NPM1 and the effect of NSC348884 on neuroblastoma cells may lead to novel treatment options for neuroblastoma patients.

ANIMAL POSTER ABSTRACTS

BIOTECHNOLOGY

A-3000

Isolation of Fibroblast-like Cells After 42 Days of Postmortem Storage of Cattle Skin in a Refrigerator. MAHIPAL SINGH and Brian Walcott. Animal Science Division, Agricultural Research Station, Fort Valley State University, Fort Valley, GA. Email: singhm@fvsu.edu

Somatic cell nuclear transfer has renewed interest in tissue storage due to its usage in reintroducing lost genetics back into the breeding pool in animal agriculture, preserving genetic diversity, and reviving endangered species. Several studies have shown an inverse relationship between cell survival and postmortem time interval. However, the limits of time interval within which live cells can be isolated from animal tissues postmortem, have not been adequately studied. The objective of this study was to assess the limits of cell survival in bovine skin stored at 4 °C after the animal death. Outgrowth of cells around skin explants (2–3 mm^2) in culture dishes was used to isolate the primary fibroblasts. Ear skin was procured from the university slaughter house from nine healthy animals and stored at 4 °C in the lab. Skin explants ($n = 90$) were cultured on eighteen 60-mm-diameter dishes (5 explants/dish) after 0, 7, 14, 21, 28, 35, and 42 days of storage (at 4 °C) in DMEM media supplemented with 10 % FBS and 50 units/mL of penicillin and 50 μg/mL of streptomycin. Outgrowth of fibroblast-like cells around the explants was scored after 10 days of culture in a CO_2 incubator. Out of 462 explants that adhered to dish surface, 282 (61.04 %) exhibited outgrowth. Our results showed outgrowth of cells in all the time points studied including 8 % explants in 42 days of postmortem storage time interval. In general, the number of outgrowing cells decreased with increasing postmortem storage time interval. To test the cytogenetic status of the cells derived from postmortem tissues stored for 42 days, we established secondary cultures from primary cells at p4 level. Cytogenetic analysis of 42 dpm tissue derived cells performed on 20 G-banded metaphase cells revealed a normal female karyotype (60, XX[20]). These cultures have been passaged up to 25 times till date and exhibit comparable morphology to controls; however, they exhibit reduced growth. These results suggest that live usable cells can be recovered from bovine skin tissues up to 42 days of postmortem, if the skin is stored at 4 °C.

CELL BIOLOGY

A-3001

Nucleophosmin (NPM1) Localization In Neuroblastoma Cells Is Disrupted By $TmPyP_4$. JEFFERY GREENLAND, Sarah Veen, Matthew Pytynia, Kelly R. Vlcek, Joshua Gasiorowski, and Kolbrun Kristjansdottir. Midwestern University, Department of Biomedical Science, 555 31st Street, Downers Grove, IL 60515. Email: kkrist@midwestern.edu

Neuroblastoma is a pediatric cancer with the high-risk form of the disease correlated with amplification of MYCN and a low 5-year survival rate. Our lab has previously found that high MYCN levels correlate with high NPM1 gene and protein levels in neuroblastoma cell lines. NPM1 is a multifunctional protein with roles in synthesis and assembly of ribosomes and DNA replication. NPM1 is primarily localized to the nucleolus, but shuttles from the nucleolus to the nucleus and cytoplasm. NPM1 protein expression has been found to be elevated in breast, prostate, and gastric cancers and in some cases is correlated with advanced disease status. Mutations in NPM1 have been identified in leukemia that alter the localization of NPM1 from nucleolar to cytoplasmic and result in more favorable overall survival. No such mutations have been identified in neuroblastoma. However, localization of wild-type NPM1 has been shown to be altered in leukemia cells after treatment with a competitive inhibitor of NPM1, $TmPyP_4$, along with an increase in cell death. The goal of this study is to examine whether $TmPyP_4$ also causes altered localization of NPM1 and cell death in neuroblastoma cells. We show that WS neuroblastoma cells treated with $TmPyP_4$ for 24 h had a dose-dependent decrease in metabolically active cells measured using a WST-1 assay. We examined endogenous NPM1 localization via indirect immunofluorescence using anti-NPM1 antibodies and DAPI stain. A GFP-NPM1 construct was transfected into neuroblastoma cells and fluorescence microscopy used to monitor localization of exogenous NPM1 protein. NPM1 was predominantly localized to the nucleolus in both endogenous and exogenously expressed NPM1. FIJI-ImageJ was used to quantify localization of NPM1 before and after treatment with $TmPyP_4$. Preliminary results indicate that in WS neuroblastoma cells, treatment with 4 μM $TmPyP_4$ for 24 h disrupts nucleolar localization of NPM1. We are currently testing the effect of $TmPyP_4$ on mutant and truncated forms of NPM1.

A-3002

Evaluating Blueberry Extracts as Nutraceutical Additives for Future Fish-feed Formulations Using a Fish Intestinal Epithelial Cell Line. V. OBEROI[1], G. S. Rai[1], G. Mahil[1], N. C. Bols[2], and L. E. J. Lee[1]. [1]University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA and [2]University of Waterloo, Department of Biology, Waterloo, ON, CANADA. Email: Vishesh.Oberoi@student.ufv.ca

The cost of fish feed is the single most expensive component in aquacuture practices and inexpensive alternative sources/ingredients are being sought. Value-added plant components are being explored as additives to provide for example, antimicrobial activity to reduce antibiotic use and to stimulate the immune system, or to enhance fillet quality and/or storability, as well as to increase value/desirability of product. Blueberries (*Vaccinium* spp.) contain health-promoting bioactive compounds such as anthocyanins (delphinidin, malvidin), flavanols (epicatechin), flavonols (quercetin), ascorbic acid, among many other compounds desirable in the food and nutraceutical industries. Evaluating the effectiveness of dietary ingredients using whole fish is costly and time consuming, thus alternative models are being sought. For the testing of novel dietary components in humans and domestic animals, cell lines have been instrumental, but a similar approach has seldomly been performed for aquacultured fish species. The salmon aquaculture industry is looking for nutraceuticals to maintain healthy stocks and supplement the dietary needs of cultured fish inexpensively. In this study, bioactive compounds present in blueberries (e.g., malvidin, delphinidin, quercetin, epicatechin) as well as crude blueberry extracts from five local cultivars (Bluecrop, Liberty, Duke, Draper, Eureka) are evaluated for their stimulatory or inhibitory effects on various cellular functions using RTgut-GC, an intestinal cell line derived from rainbow trout. Cell viability, monolayer integrity, phagocytosis, migration ability, and collagen synthesis were evaluated to assess differences in bioactivity since fruit quality, antioxidant capacity, and flavonoid content have been reported to vary among these cultivars.

CELLULAR AND MOLECULAR TOXICOLOGY

A-3003

Evaluation of Bioactivity of Blueberry Extracts from Organic and Conventional Cultivars Using Fish Cell Lines. G. MAHIL[1], V. Oberoi[1], K. Moghrabi[1], G. S. Rai[1], N. C. Bols[2], and L. E. J. Lee[1]. [1] University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA and [2]University of Waterloo, Department of Biology, Waterloo, ON, CANADA. Email: Gaganjeet.Mahil@student.ufv.ca

Fish feed (based on costly fish meal components) and antibiotics are the most expensive factors affecting the

viability of salmonid aquaculture practices. Inexpensive plant-based feed components are being sought to add to fish feed as nutraceuticals, that is, not only for its nutritional needs for the fish but also as pharmaceutical components to enhance the health of fish (and also for the consumers, ie humans) as well as for marketability purposes (enhance taste, color, value). For humans and land-based animals, the nutritional and/or pharmacological value of food extracts have been routinely evaluated using in vitro methods employing intestinal cell lines. A similar approach for evaluating feed components for aquacultured species have not been reported. In this study, we explore a non-traditional plant product for fish feed: Blueberry extracts, as an inexpensive source of nutraceuticals for fish. Blueberries are rich in antioxidants and micronutrients, thus are valued for human and animal consumption. We evaluate organic vs. conventionally grown blueberries to assess differences in bioactivity since the fruit quality, antioxidant capacity, and flavonoid content have been reported to vary depending on the crop cultivation method. Furthermore, the persistence of systemic pesticides (pesticides that are taken up by plants and remain active within the plant tissues) such as neonicotinoids (NN), questions not only the nutritional value but the safety of conventionally grown blueberries that are regularly exposed to insecticides. Effects on cellular functions, antioxidant activity, and cell integrity using RTgut-GC, a rainbow trout intestinal cell line, and other relevant fish cell lines will be presented.

A-3004

An Assessment of the Sensitivity of the Bovine Lens Assay as a Model for Ocular Toxicity. DAVID J. MCCANNA[1], Manlong Xu[2], and Jacob G. Sivak[2]. [1]University of Waterloo, Centre for Contact Lens Research, Optometry and Vision Science, Waterloo, ON, CANADA and [2]University of Waterloo, Optometry and Vision Science, Waterloo, ON, CANADA. Email: djmccann@uwaterloo.ca

Purpose: The maximum tolerated dose in the eye of an ophthalmic chemical is affected by the concentration of the chemical in the eye drop, the duration of contact, and the total dose instilled. Using the bovine lens, an in vitro model that is capable of evaluating both single and multiple-dose exposures was developed. Methods: Bovine lenses were exposed to benzalkonium chloride (BAK), 0.01 % in 0.9 % sodium chloride solution, for 15 min. Each bovine lens was then rinsed, placed into fresh medium, and incubated at 37 °C in a CO_2 incubator. The single-dose group was maintained in culture medium after initial exposure for 96 h. The multiple-dose group was exposed for 15 min to BAK (0.01 %) solution 24, 48, 72 h after the initial exposure. On the fourth day of incubation of the lenses, the metabolic activity was assessed using the alamarBlue viability assay. In addition, the optical quality of the lenses was determined using a laser scanner. Results: Single exposure of 0.01 % BAK caused the metabolic activity of the bovine lens to significantly decrease 33 % ($p < 0.05$) 4 days after the initial exposure when compared to untreated control lenses. Treating the bovine lens with multiple treatments of 0.01 % BAK caused an even greater drop ($p < 0.05$) in metabolic activity to less than 73 % of the control. Using the laser scanner, the toxicity of the BAK treatments was also detected as both the single and multiple exposed lens groups showed significantly reduced optical quality compared to the control lenses ($p < 0.05$). This drop in metabolic activity also occurs after exposing cultured human ocular cells to BAK. Conclusion: BAK is a chemical that has been shown in vivo to be toxic to the eye at 0.01 %. Using this in vitro assay, we were able to demonstrate that BAK toxicity could be determined. In addition, a repeat exposure to 0.01 % BAK over the course of 3 days caused increased toxicity and demonstrates that this assay can be used to measure the toxicity of ophthalmic chemicals that are used with multiple installations over the course of many days.

A-3005

Evaluation of Sub-lethal Effects by Neonicotinoids in Fish Cell Lines. K. MOGHRABI, V. Oberoi, G. S. Rai, H. Sidhu, and L. E. J. Lee. University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA. Email: Kamal.Moghrabi@student.ufv.ca

Novel pesticides have gained regulatory acceptance as they are touted to be specific to target pests through known modes of action. However, non-lethal and non-specific effects could occur in non-target animals through as yet undescribed mechanisms. Neonicotinoids (NNs) have become the most widely used insecticides in agricultural practices worldwide, and although, advertised as safe for non-target animals and specific against insect pests, chronic and sub-lethal effects are beginning to become apparent in mammals, birds, and aquatic animals. Unlike previous generations of pesticides, NNs are taken up within plant tissues and are long lived not only systemically within treated plants or animals, but also can persist in soil and water bodies for several years. This persistence in the environment and its ability to permeate within the tissues of plants and animals exposes vertebrates to

cumulated doses that could have detrimental effects. Since significant amounts end up in water bodies, effects in freshwater and/or estuarine fish are specifically worrisome. Evaluating various fish species at the whole organismal level is costly and prohibitive thus representative fish cell cultures could provide initial insights into the mode of action of NNs. Cell cultures are excellent investigative tools to study biological functions in isolation without compounding effects of physiological processes within whole organisms. Cell lines from various fish species: trout, eel, walleye, killifish, fathead minnow, were evaluated for effects on cell migration, cell proliferation, and wound closure using 'scratch assays' that are easy to perform and score. For all cell lines, inhibition of cell migration with increasing NN doses was observed.

DEVELOPMENTAL BIOLOGY

A-3006

Development of a New Cell-based Assay Using Human ES/iPS Cell-derived Neural Stem Cells for Developmental Neurotoxicity (DNT) Testing. MIKA SUGA[1], Hiroaki Kii[2], Takayuki Uozumi[2], Yasujiro Kiyota[2], and Miho K. Furue[1]. [1]National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, JAPAN and [2]Nikon Corporation, Tokyo, JAPAN. Email: mikasuga@nibiohn.go.jp

Human pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, could be a promising new tool for in vitro developmental neurotoxicity (DNT) testing. Several DNT testing methods based on human ES cells have been proposed with "3R" concept refining, reducing, and replacing the DNT tests on vertebrata. However, animal-derived components such as Knockout Serum Replacement (KSR), B27 supplement, or Matrigel are still used to derivate neuronal cells from human ES cells in the methods. Recent studies have suggested that evaluation using the comprehensive transcriptomics analysis is applicable to detect gene expression response to chemical exposure. However, more cost-effective, rapid, and simple screening method would be expected. To address these issues, we are developing a new ES/iPS cell-based assay for DNT testing under completely chemically defined culture condition without any animal-derived components using an imaging analyzer. We used an antiepileptic drug, valproic acid (VPA), which is known to cause neural tube defects, to verify the results measured by our method for DNT testing. The effects of VPA on the commercially available neural progenitor cells or neural stem cells differentiated from ES/iPS cells in our new culture conditions were assessed by immunohistochemical analysis or phase-contrast imaging. The imaging analysis data have provided more detail information about the effect of VPA. Our cell-based assay could be useful for cost-effective, rapid, and simple DNT testing.

GENE THERAPY

A-3007

A Novel Technique to Quantify Medium- to High-throughput Live Cell Transgene Expression Over Time. S. C. VEEN and J. Z. Gasiorowski. Midwestern University, Department of Biomedical Sciences, 555 31st Street, Downers Grove, IL 60515. Email: scveen@midwestern.edu

Despite improvements in methodology and equipment, there are still several well-established, but limited, traditional techniques that are commonly used today to study and quantify transgene expression. One of these techniques focuses on using microscopes to measure fluorescent reporter transgene expression. Although this technique can be useful in observing single cell transgene expression, large cell populations cannot be observed as a whole unless time-consuming stitch image acquisition is performed. Other existing methods involve the use of secreted reporter genes, but they typically require multi-step assays (fluorescence/absorbance/luminescence) to be carried out to measure the expression level. While these methods can deliver results, they can also be strenuous and easily subjected to user bias. To improve upon these existing techniques, the aim of our research was to test if a macro-scale fluorescence photo-imager would allow us to non-invasively acquire sensitive, quantitative, single snapshots of an entire population of cells in a multi-well plate for reporter analysis. Normally, a macro-scale fluorescence photo-imager is designed to detect mm-sized bands on agarose or polyacrylamide gels; however, with appropriate light sources and filters, we used it to image multi-well tissue-culture plates seeded with a range of cell densities. The fluorescence reporter expression of the entire population of cells was then tracked at various time points between 4 and 72 h. We observed that the transgene expression level demonstrated a linear trend in relation to the number of cells. This model can serve as a powerful, efficient tool to quantify live cell transgene expression over time in a medium to high-throughput fashion and allow us to study the expression of transgenes or transfection reagents on a macro-scale level.

ONCOLOGY

A-3008

Cul5 Knockdown Increases the Viable Cell Number in MDA-MB-231 Breast Cancer Cells: Implications for Hsp90 Inhibitor Chemotherapy. LAUREN A. C. ALT, Parvaneh Akbari, Shubha Mathur, Joseph Rojas, Erik Mersereau, Kolbrun Kristjansdottir, and Michael J. Fay. Midwestern University, Department of Biomedical Sciences, 555 31st Street Downers Grove, IL 60515. Email: mfayxx@midwestern.edu

The *Cul5* gene is located on a region of chromosome 11 that is associated with LOH in breast cancer, and as a result, Cul5 is implicated in breast tumorigenesis. Inhibitors of HSP90 are being evaluated as novel chemotherapeutic agents. The mechanism of action of these drugs involves the recruitment of Cul5 to the HSP90 complex which results in the degradation of HSP90 client proteins by the ubiquitin-mediated proteasome pathway. One such client protein that Cul5 degrades is the oncogenic epidermal growth factor receptor HER2/ErbB2. Triple negative breast cancer is an aggressive form of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR), or HER2/ErbB2. The goal of this project is to determine the effects of Cul5 knockdown on the MDA-MB-231 triple negative breast cancer cell line, and to determine if MDA-MB-231 cells are responsive to the HSP90 inhibitor 17-AAG. Cul5 knockdown was performed by transfecting MDA-MB-231 cells with Cul5 targeting siRNA pool or a non-targeting negative control siRNA pool. Cul5 knockdown was verified 72 h post transfection by Western blot analysis, and cells counts were performed using Trypan blue and the BIO-RAD TC20™ automated cell counter. Cells were also treated for 72 h with vehicle control (DMSO), or with 0–1500 nM 17-AAG, and cell counts were performed. Knockdown of Cul5 expression at the protein level was successful as demonstrated by Western blot analysis. The Cul5 knockdown samples demonstrated an increase in the number of viable cells compared to the non-targeting negative control siRNA samples. The 17-AAG treated MDA-MB-231 breast cancer cells demonstrated a dose-dependent decrease in the number of viable cells without a change in the percent viability. Since MDA-MB-231 cells do not express HER2/ErbB2, we are currently performing experiments to identify the degraded HSP90 client proteins that are contributing to the chemotherapeutic effect of 17-AAG in triple negative breast cancer. We are also performing experiments to determine if Cul5 knockdown attenuates the chemotherapeutic effect of HSP90 inhibitors.

A-3009

Osteogenic Marker Gene Expression of Osteoblasts Cultured in Two and Three Dimensional Environments. IMAN MOHAMED, Elisha Pendleton, and Nalini Chandar. Midwestern University, Department of Biochemistry, 555 31st Street, Downers Grove, IL. Email: imohamed88@ midwestern.edu

Osteosarcoma is the most common primary malignant bone tumor in adolescents and young adults with tumors occurring in areas where the bone is growing rapidly. Loss of differentiation in osteoblasts holds prognostic significance in osteosarcoma patients. Osteosarcomas are frequently deficient in classic tumor suppressors such as p53 and retinoblastoma (Rb). Understanding the normal process of osteoblast differentiation and the role Rb plays in its progression helps us understand the consequences of deviating from it. We have utilized MC3T3E-E1, a murine osteoblast-like cell line that has been extensively used to study osteogenesis in vitro. We have cultured these cells in a three-dimensional environment, and analyzed their differentiation capacities. We have also studied the loss of Rb function on differentiation capacity in 2 and 3D cultures using the MC3T3-E1 cell line with a stable knockdown of Rb expression. Levels of known osteogenesis markers such as Runx2, Osterix, BSP, and Osteocalcin have been analyzed using real-time PCR. For mineralization changes, a quantitative Alizarin Red assay was utilized. Growth characteristics of cells grown on collagen beads was also studied. Our data shows that culturing osteoblasts in 3D demonstrated differentiation earlier and at a higher level as determined by the expression of markers of bone differentiation. Runx2, Osterix, BSP, and osteocalcin showed the expected response to a differentiation promoting media, while loss of Rb either reduced or changed the pattern of expression of these markers. There was roughly a three-fold increase in calcium deposits in wild-type cells when compared to their mineralization in 2D cultures. With Rb loss, mineralization capacity was reduced fourfold when compared to controls. In conclusion, our results show that growth in 3D hastens osteoblast differentiation and expression of bone markers when compared to their traditional 2D growth in cultures. Rb deficiency in osteoblasts causes a decrease in expression of bone markers in both 2D and 3D cultures when compared to controls.

SIGNAL TRANSDUCTION

A-3011

NPM1 Protein-Protein Interactors in Neuroblastoma: Decoding the Domains. ALEJANDRO ALVAREZ[1], Jaclyn Campbell[1], Tyler Johnson[1], Collin Hickey[1], Andy Truman[2], and Kolla Kristjansdottir[1]. [1]Midwestern University, Department of Biomedical Sciences, Downers Grove, IL and [2]University of North Carolina, Department of Biological Sciences, Charlotte, Charlotte, NC. Email: kkrist@midwestern.edu

NPM1, also known as nucleophosmin, is a nucleolar ribonucleoprotein with multiple functions within cells including involvement with ribosome biogenesis, molecular chaperoning, centrosome duplication, and genomic stability. NPM1 consists of multiple domains including the N-terminal domain, which is involved in oligomerization and molecular chaperoning, and the C-terminal domain, which is involved in DNA/RNA binding. NPM1 dysregulation has been shown to be involved in human cancers, such as ovarian and gastric cancer as well as leukemias. In some cases, elevated levels of NPM1 correlate with advanced disease. Neuroblastoma is a childhood cancer arising from neural crest cells, which most often presents as a tumor on the adrenal gland. We have found elevated levels of NPM1 protein in high-risk (MYCN positive) neuroblastoma cell lines. The high-risk form of neuroblastoma has poor survival rates for patients, indicating a need for new treatments. NPM1 is known to interact with a number of different proteins in order to carry out its functions within the cell, but these interactions have not been studied in neuroblastoma. To investigate protein-protein interactors of NPM1 in neuroblastoma, a yeast two-hybrid screen was done using full length NPM1 protein as the bait and a human fetal kidney library of proteins as prey. Hits from the screen were re-transformed back into yeast in order to perform β-galactosidase assays to determine the strength of the interaction. Selected hits from the screen using the full length NPM1 were also assayed against truncated forms of NPM1. These truncated forms had one or more domains of NPM1 removed. Interaction strengths determined by the β-galactosidase assays were normalized to the strength of interaction using the full length NPM1. Through this method, we are able to correlate protein binding of NPM1 interactors to domains within the protein and quantitate binding strength.

EDUCATION POSTER SESSION

E-3000

Training the Next Generation-Y Through Innovative and Experiential Science Curricula, and Professional Development. Marceline Egnin[1], OSAGIE IDEHEN[1], Gregory Bernard[1], Steven Samuels[1], Desmond Mortley[1], Franklin Quarcoo[1], Conrad Bonsi[1], Olga Bolden-Tiller[1], iBREED Students[1], and Craig Yencho[2]. [1]Tuskegee University, College of Agriculture, Environment and Nutrition Sciences, Tuskegee, AL 36088 and [2]North Carolina State University, College of Agriculture and Life Sciences, Department of Crop Science and Production, Raleigh, NC 27695. Email: megnin@mytu.tuskegee.edu

Engaging minority students with in vitro sciences experts was the realm of the "Innovative Plant molecular Breeding Research and Experiential Education" (iBREED) project as a contribution to training in response to the "Dying Breed" concept of students shying away from plant sciences, identified by USDA-NIFA in this big data era. In a discovery, creative, and imaginative manner, the Tuskegee University iBREED program helps students envision themselves as the next generation of plant breeders, changing the world one genome at a time. A cohort of 18 iBREED students were brought together in a collaborative environment working in teams of peers. Student mentees carried out hands-on plant-based research and gained first-hand knowledge from distinguished scientists in true conventional research as well as modern molecular breeding techniques including tissue culturing methods, bacterial and plant transformation, seed development, genetics, and plant biotechnology. In addition, students benefitted from visits and interactive engagement with experts in CRISPR technology, government agencies, and agricultural corporations. Modest financial incentives played an important role in recruiting students, sustaining their drive to master the iBREED training, and molding professionals in precision breeding and in vitro plant science. The success of this project in near-peer mentors role helped overcome students' indifference and leverage research opportunity for their next generation career; thus, fostering a new appreciation for the meaning and relevancy of plant breeding and related precision sciences. *Work supported by*: *USDA-NIFA 1890-CBG funded iBREED project to Tuskegee University Plant Biotech Lab*, *GWCAES-CAENS*.

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