Identification and Characterization of Exosomes from Cardiac Fibroblasts in Ossabaw Pigs with Metabolic Syndrome and Diabetes

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Introduction:

Cardiovascular disease (CVD) is the leading cause of death worldwide, and many patients with diabetes will develop CVD. However, there is no current method to detect CVD, and many patients are unaware of their illnesses until catastrophic events like heart attacks and strokes occur. A biomarker – a measurable substance that indicates the body's condition – for CVD in diabetic patients could aid in diagnostics and treatment of CVD before a potentially fatal event arises. This study searches for such a biomarker on vesicles (exosomes) secreted by cardiac fibroblasts. Recent research shows that healthy cells produce different exosomal RNAs and proteins than diseased cells.

Methods:

Cardiac fibroblasts of Ossabaw swine were used to produce the exosomes. Not only do Ossabaw pigs have a natural predisposition for obesity and metabolic syndrome (MetS), which leads to diabetes, but they also posses a cardiovascular physiology and pathology similar to humans. The pigs were either fed a normal diet (control) or a high calorie, high trans-fat diet (MetS) for 8-9 months. The heart tissue was minced, rinsed with saline, and underwent collagenase digestion before selective attachment to tissue culture plastic, yielding pure fibroblast culture. The cultured cells were serum-starved before exosome isolation by centrifugation, filtration, and ultracentrifugation.

Results:

Exosomes were purified from the conditioned media of primary cardiac fibroblasts of male MetS/diabetic pigs and healthy littermates. Transmission electron microscopy demonstrated that the exosomes exhibited characteristic cup-shaped morphology. Western immunoblotting was performed to confirm the presence of exosomes.

Conclusion:

Based on morphology, size, and protein composition, we defined the release of exosomes from primary cardiac fibroblasts and provide their first extensive characterization. Future studies will compare the differences of exosomal protein profiles from cardiac fibroblasts of diabetic and healthy pigs by two-dimensional gel electrophoresis (2-DGE) and mass spectrometric analysis.

Death comes to all people; this is one of life's few certainties. But premature death comes to many, even though it can many times be prevented (given that the cause is known and avoidable). However, if the cause is not know, it is not so easy to avoid it, and that which is treatable becomes fatal. Cardiovascular disease, for example, is a cause that is often unknown until death is near. As a result, heart disease is the number one cause of death in the United States and worldwide.

I have investigated such matters as the cost of the disease in both dollars and lives, its incredible commonality, and its correlation with diabetes and the costs associated with diabetes. I have also researched the regional aspects, including why it is so important to the US and to Mississippi, as the disease in these areas are compounded by cultural factors, like diet and inactivity that lead to medical risk factors, like obesity, hypertension, and diabetes.

People do not know that they have heart disease because there is no singular test for it. There are general symptoms and correlations, but there is no standard procedure that can be performed in a primary care setting that is even close to definitive. Obesity, diabetes, hypertension, and high cholesterol are risk factors - but these are correlations, and are not sufficient to diagnose heart disease alone. (Cholesterol, for instance, can change significantly throughout the day depending in diet, and obesity is a symptom of many other pathologies.) The kind of imaging techniques currently used to diagnose heart disease are time-consuming, uncomfortable, and expensive, so patients and their primary care physicians often will not use them unless the disease has already manifested itself - and by the time a heart attack or stroke occurs, it is often too late for the patient.

It is desirable, therefore, to develop a test for heart disease that could be used efficiently, quickly, and with minimal patient discomfort. The purpose of this study is to do just that: to develop a test for heart disease so that it could be treated before these deadly events occur. Specifically, the goal is to find an exosomal biomarker using the most high risk population: the diabetic and the obese.

The animal model for this study is the Ossabaw pig, a swine known for its genetic predisposition to heart disease and diabetes. (In fact, in the wild, the pigs become diabetic - and normal again - during feeding and fasting cycles over the course of a year.) These pigs are perfect for this study because they are a similar size to humans and have a similar cardiovascular system in anatomy, physiology, and pathology. The pigs are treated for eleven to twelve months in either a control (normal, 2,400 kcal/day) or a metabolic syndrome (4,500 kcal/day) diet. The obese pigs being used in this study were not only double the weight of the control pigs, but their blood pressure was also much higher, and the cholesterol levels were nearly four times that of the control pigs.

The hearts of the pigs are then shipped to Mississippi from Indiana, where they are minced, rinsed with saline, and digested with collagenase. This separates the cells from the extracellular matrix. Selective attachment to tissue culture plastic is then used to separate the cardiac fibroblast cells from the others. These cells are most likely to secrete the exosomes needed for the study, since they create the extracellular matrix. These cells are cultures and weaned off of serum (since serum contains exosomes) but they are never split; they need to be as similar to the original pathology of the heart as possible.

(Although I did some work with the cell culture, most of my activity begins here, and continues until the very end of the process, in which I am actively involved.) The serum-free conditioned media is then pipetted off the cells, and is centrifuged several

times: once at 2,000g to remove debris, once at 10,000g to remove any whole cells, it is filtered at 0.22 micrometers, and is lastly centrifuged for two hours at 100,000g, each time removing the media supernatant. This is repeated until all the conditioned media gathered for that event is used. Then, it will be washed to remove contaminating proteins and centrifuged again for two hours and 100,000g to finally have a clean exosome pellet.

So, what are exosomes? Exosomes are tiny membrane-bound vesicles that cells secrete as a method of intercellular communication. These protein and nucleotide-containing compartments are a new and active area of research right now in several pathologies because the healthy cells secrete exosomes with different contents than unhealthy cells do; therefore these exosomal contents can be used as biomarkers for disease. They are also being explored to further understand cellular function and communication among cells in general.

They are of great clinical importance because exosomes are secreted into bodily fluids, which means that they are in the blood, the urine, the cerebrospinal fluid, and even the saliva of patients. Therefore, they could potentially be gathered without the time, expense, and discomfort of current tests. If the test could be done so easily, then it could be added to a metabolic panel and performed at yearly checkups in a primary care setting.

In addition, exosomes outperform current tests for cardiovascular disease because they are more predictive and more specific. For example, instead of merely testing positive for cancer, the exosomes can inform the physician and patient of the current stage of cancer and metastatic probability - two things that greatly influence treatment. In addition, they can be used to monitor other treatments, and may one day even be a treatment themselves: by targeting exosome contents or adjusting the amount of exosomes secreted, all of the downstream pathways (including gene transcription in the target cell) would be affected. One must keep in mind that, since healthy cells secrete exosomes as well, their presence and their contents are not necessarily detrimental to the organism - some exosomes may in fact be beneficial.

To prove that the exosomes exist, however, is a matter to itself. Since exosomes are on the nanometer scale, the aforementioned exosome pellet is often not visible even when hundreds of milliliters of media are centrifuged into one sample. Therefore, they must be viewed under the transmission electron microscope. The exosomes found were of the proper size (around 30-150 nanometers in diameter) and displayed the cup-shaped morphology expected of exosomes.

To further prove that exosomes were obtained, a Western immunoblotting technique was performed on both exosomes and whole cells. Using cluster of differentiation protein 63 (CD63) and heat shock protein 70 (HSP70), the cells (for HSP70, and barely, CD63) and exosomes (both) showed banding. This is the pattern expected. Had the exosomes not had these markers, they would not be classified as exosomes, since all known exosomes have these membrane proteins. It is expected to see these in cells as well, especially HSP70, which is fairly ubiquitous in membranes. CD63 is only found in cell membranes occasionally, which explains its light, barely-there banding.

In the future, tests will be run to determine the contents of the exosomes. This is the most important part. If the exosomes of the diseased cells contain different proteins, RNA, or DNA than the controls, it may just be the biomarker needed to test for heart disease. We will do this using 2-dimensional electrophoresis and mass spectrometry. To

be sure that these exosomes are secreted in a manner that could be used clinically, the same procedure will be run on the blood exosomes to make sure that the results match (otherwise, the test would involve heart surgery, which would nullify the entire point of the study). If it this is the case, a test for heart disease could be on its way to implementation in practices worldwide, and heart disease can be treated before lives are at risk - or worse, lost.

I have been a part of this project in many ways, from cell culture to exosome isolation to biochemical assays. I have learned things that are specific to biochemistry, like running a Western Blot or using the autoclave, and things that will carry over into other mediums, like aseptic techniques and keeping my hands almost robotically steady. The only thing I have never played a part in is raising the pigs, which are at the medical college in Indiana. I have done research on other articles as well, summarizing and organizing them and preparing them to be sourced in a publication. This includes the papers mentioned earlier, on such topics as national as well as out-of-pocket costs and death rates and comorbidity, but includes many more on exosomes and their activities. Exosomes, as it turns out, have been implicated as biomarkers for several different kinds of cancer as well as neurological disorders, liver diseases, and kidney diseases. Since their mechanisms of exiting the cell and entering another are still not fully elucidated, some research is being done on these aspects. Others are studying them even as potential causative agents of certain disease symptoms, or even disease progression.

I have presented the findings thus far at the Biology Undergraduate Research Symposium (BURP) in the form of a poster that I created and at the Honors Undergraduate Research Program Symposium (HURP) in the form of a speech and powerpoint. Although I did not win anything at BURP, at HURP I was awarded first place in the "biological sciences and engineering talks" category.

Although the project is far from finished, the first step has indeed been achieved. More samples will be needed before a proper paper can be published, but it has been a running start, so to speak. Doing the symposia has not only improved my general speechwriting and speech-giving skills, but also improved my ability to take something incredibly specific and incredibly technical and put it in terms that people unfamiliar with it can understand. This is an important skill in many aspects of life, even outside the professional world. I have also learned to think well on my feet, to answer questions with poise and confidence, and developed a deeper understanding of the project for myself through these presentations.

As for my own experiences and how this applies to me and my future, I have found it invaluable. Yes, my hands are surgically steady, and yes, I know a lot more about the actual act of doing science, but my learning experience goes far beyond how to do 2-dimensional electrophoresis, use a pipet, or culture cells. I do not think the same way as I did before starting research. I'm more open and more closed at the same time - open to all the possibilities and creative solutions, but less gullible and more likely to fact check everything I hear from several sources, be it in a scientific journal or on the news at night. I am skeptical of everything, but cynical about nothing.

On a more intimate level I also feel a driving sense of purpose. It is wonderful to be part of something so much larger than my own life, something that has the potential to improve the lives of people not just in Mississippi, not just in the US, but worldwide. Millions of people die of heart disease ever year. Billions of dollars are spent on

treatment in the US alone. And as the population ages, eats less healthy and stay more sedentary, death from this disease is increasing - and will continue to, unless solutions are found. To be a part of that solution is priceless.

Although I do not plan to pursue a Ph. D, I do believe that this experience will be helpful to me as an M.D. As a physician, I need all of these skills, from the calm demeanor and quick thinking to the creativity and analytical abilities to the greater understanding of science and how it contributes to patient care. I see better now how the whole of physiology is interdependent - which affects how one must conduct biochemical investigations and treatments. In addition, this has somehow made me more compassionate. It can be easy for people to blame the patients for diseases connected to lifestyle choices, but that is not our place as scientists or physicians to pass moral judgment. We exist to serve, to treat, to discover, not to condemn, especially with so much conflicting information in the media about what is healthy. We must assume an objective role.

I will be a better doctor for having done this, and will likely remain active in research from the medical side, merging what happens in the lab (the theoretical) with what happens in the clinic (the practical) - a gap that seems to get wider and wider as technology advances and legislation passes.

I look toward next year with excitement, glad that I have been afforded the opportunity to participate in this project for another year. Maybe this next year, we will have a publication. This has become so much more than a slot on my resume. It's changed me as a scientist and as a human being. My only regret is that I will likely not be able to see this project to its end, as that is many years away, and I have but one year left at State. The chance to be a part of something that could save so many lives is a priceless gift.

Death may come to all of us, but that doesn't mean we should hasten its arrival. And if we succeed, we may postpone it for some people for a few more years.

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Background

Cardiovascular disease (CVD) is the leading cause of death in the US and worldwide[2,4]. In addition, type II diabetes mellitus is often comorbid with CVD: in fact, 65% of patients with type II diabetes die of CVD[1]. However, since there is no current method to detect CVD, most patients are unaware of their condition until catastrophic events – like heart attacks or strokes – occur. A biomarker – a measurable substance that indicates the body's condition – for CVD could improve diagnostics and treatment of CVD before a potentially fatal event arises.

Exosomes are nanometer-sized vesicles secreted by cells as a communication mechanism. Recent research shows that healthy cells produce different exosomal RNAs and proteins than diseased cells. Therefore, exosomal biomarkers may be just the kind of diagnostic tool that diabetic patients need to detect CVD.

The exosomes for this study come from Ossabaw swine. Ossabaw swine have genes that predispose them to obesity, metabolic syndrome, and diabetes[3]. They also have a cardiovascular system that is similar to humans in anatomy, size, and pathology, making them good candidates for CVD study[3].

"THRIFTY GENOTYPE" (OBESITY)

ACTIVE LEAN **INACTIVE OBESE**

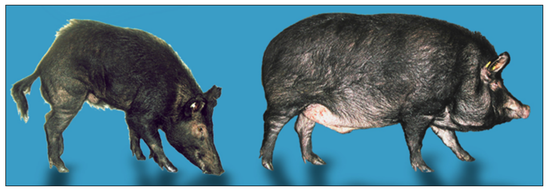


Figure 1 The phenotype of Ossabaw Swine. Lean, left; MetS, right.

LARGE ANIMAL MODEL MetS

	Ossabaw Swine
1. Obesity	Yes
2. Insulin resistance	Yes
3. Glucose intolerance	Yes
4. Dyslipidemia (↑LDL/HDL)	Yes
5. Dyslipidemia (↑TG)	Yes
6. Hypertension	Yes

Sturek et al. In *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*, 2nd Ed. M. Swindle (Ed.). p. 397, 2007
Lee et al. *Hepatology* 50:56, 2009
Edwards, Neeb et al. *Cardiovasc. Res.* 85:631, 2010

Objectives

1.To characterize exosomes in control and diabetic male Ossabaw swine based on morphology and Western immunoblotting.

2. Once characterized, exosomes may then be analyzed for the presence of proteins and miRNA that could serve as biomarkers for CVD in cases of diabetes.

Methods

Preparing the Pigs

- Control pigs (2) were fed a standard 2400 kcal/day diet for 8-9 months[3].
  - 22% protein
  - 70% carbohydrate
  - 8% fat
- MetS pigs (1) were fed a high-fat 4500 kcal/day diet for 8-9 months[3].
  - 13% protein
  - 40% carbohydrate
  - 47% fat (2% cholesterol)

Isolating the Cells

1. Cardiac tissue from left ventricles was minced and rinsed with saline.
2. Tissue was then digested with collagenase to detach cells from extracellular matrix.
3. Cardiac fibroblasts were isolated by selective attachment to tissue culture plastic.
4. Primary fibroblasts were cultured and serum-starved before exosome isolation.

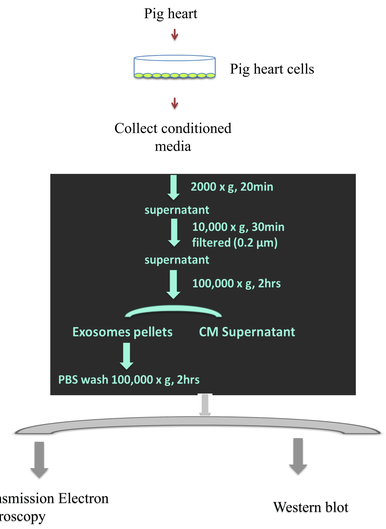
Isolating the Exosomes

Exosomes from the CM of SCN2.2 were purified as described as in flow chart.

Analysis

Transmission electron microscopy and immunoblotting analyses were performed.

Flow chart below for the exosome purification procedure based on differential ultracentrifugation and assays:



Results

Table 1. Metabolic Syndrome characteristics

Treatment Group	Body Weight (kg)	Total Cholesterol (mg/dL)	Blood pressure (mm.Hg)
MetS	94	275	155/98
Lean	45.5	72	130.5/70

Transmission Electron Microscopy

A B

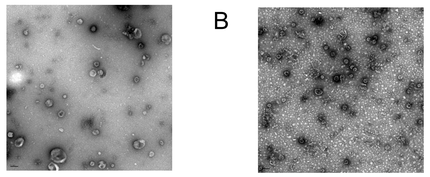


Figure 2: Representative photomicrographs illustrating the morphological properties of cardiac fibroblasts – derived exosomes and MetS (A) from Lean Control (B) of Ossabaw swines by Transmission Electron Microscope (TEM). TEM revealed vesicles of about 30-150 nm, consistent with sizes of exosomes. Scar bar = 100 nm.

Western Immunoblotting

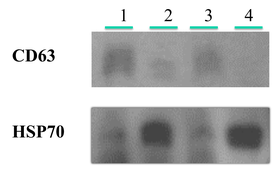


Figure 3. Biochemical characterization of exosomes by western blot. Protein (10 µg) from cardiac fibroblasts – derived exosome (1, 3), cell lysates (2, 4) from lean (1,2) and MetS (3,4) pigs are separated on 10% SDS-PAGE and analyzed by immunoblotting. Western blot analysis shows immunoreactive bands for CD63 and HSP70, two of well characterized exosome markers.

Conclusion

Based on morphology, size, and protein composition, the release of exosomes from primary cardiac fibroblasts of Ossabaw swine was defined and characterized. They displayed cup-shaped or round morphology as well as the nanometer scale size that is expected of exosomes. Exosomes were positive for common exosome proteins HSP 70 and CD63, further confirming their identity. Future studies will compare the differences in exosomal protein profiles of MetS/ diabetic and healthy Ossabaw cardiac fibroblasts by two-dimensional gel electrophoresis (2-DGE) and mass spectrometric analysis.

Acknowledgements

[1]American Diabetes Association. (2015). *Heart Disease*. Retrieved from http://www.diabetes.org/living-with-diabetes/complications/heart-disease/.

[2]Centers for Disease Control and Prevention. (2015 Feb. 6). *Heart Disease*. Retrieved from http://www.cdc.gov/heartdisease/index.htm.

[3]Sham JG, Simianu VV, Wright AS, Stewart SD, Alloosh M, Sturek M, Cummings DE, and Flum DR. Evaluating the Mechanisms of Improved Glucose Homeostasis after Bariatric Surgery in Ossabaw Miniature Swine. 2014. Journal of Diabetes Research, 2014 (2014). DOI: 10.1155/2014/526972.

[4]World Health Organization. (2014 May). *The Top 10 Causes of Death*. Retrieved from http://www.who.int/mediacentre/factsheets/fs310/en/.

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Characterization of exosomes from cardiac fibroblasts with metabolic syndrome and diabetes

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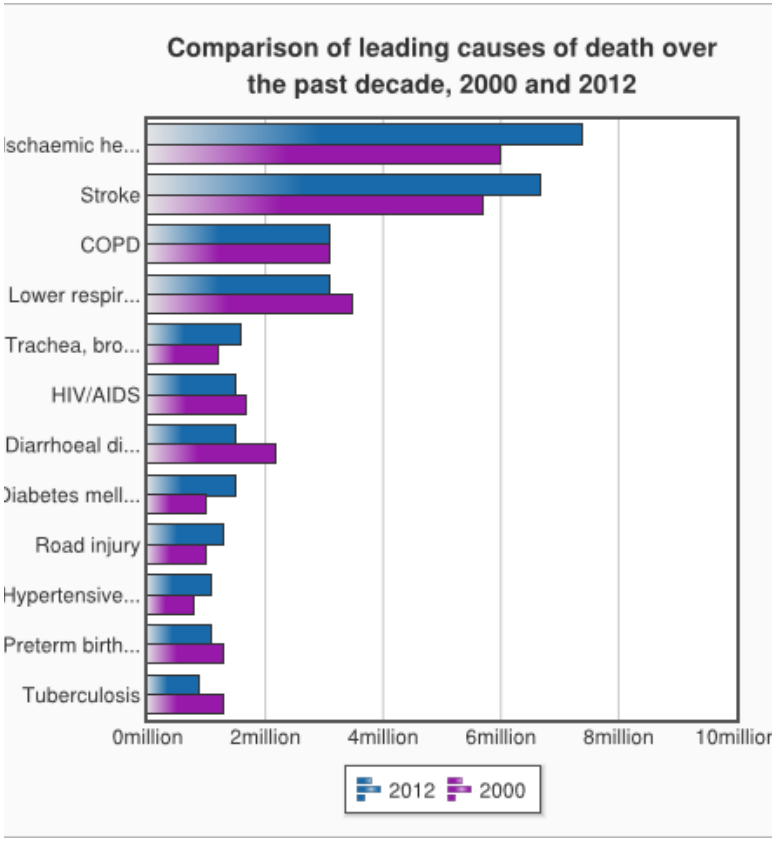
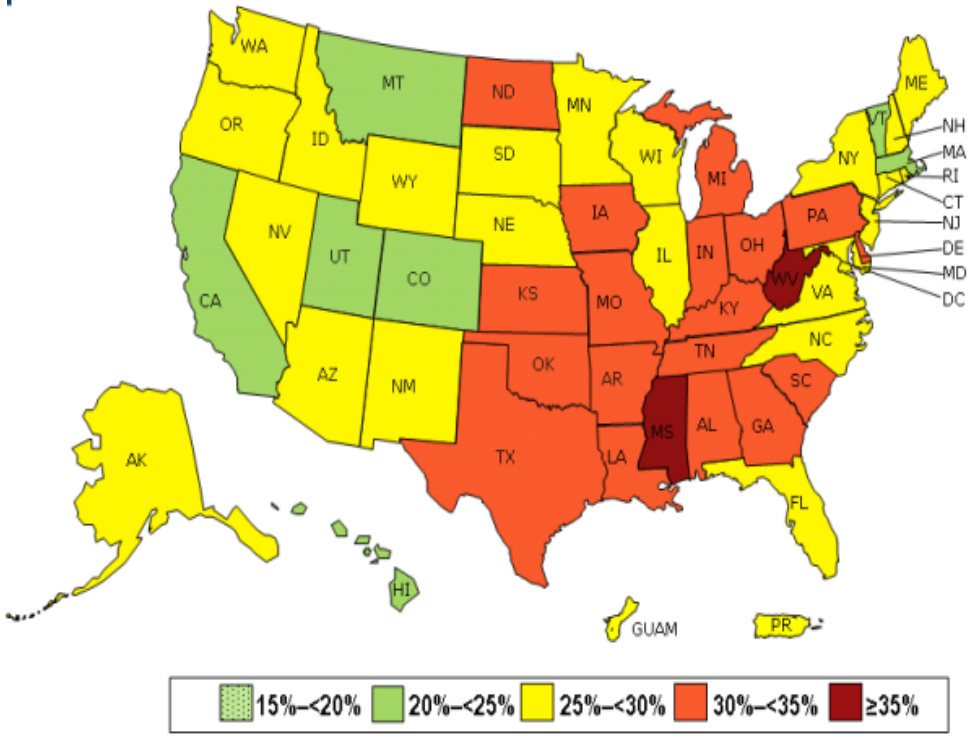
A Growing Problem

- CVD kills more people than any other disease in the US and worldwide[1,2]
- 3 in every 10 deaths in the world is from CVD[2]
  - ¼ of all US deaths[1]
- Almost 10% of the US population has diabetes[3]
  - Diabetics are at a higher risk for CVD
- About 65% of diabetics die from heart disease[4]



Our Home

- Mississippi has the highest rates of diabetes (11.7% of population) in the US[3]
- Mississippi is tied with West Virginia for highest obesity rates at 35.1% of the population[5]



Cost of Diabetes and CVD

- A **single, survived** heart attack costs over $70,000 for the first 36 months of care[6]
  - About 735,000 people in the US have heart attacks every year[2]
- Out of pocket costs for families of patients with non-communicable diseases:
  - 2% to 158% of household income in a worldwide study[7]
- The ADA estimates the total cost of diabetes in the US alone is $245 billion per year[8]
  - $69 billion in reduced productivity
  - $176 billion in medical costs
- 30-45% of the cost of care for Type II DM are due to complications of the disease

Exosomes: The Basics

- Nanometer-sized vesicles secreted by cells
- Used for communication
- Contain variety of contents:
  - Small RNAs
    - Esp. microRNA
  - DNA
  - Proteins

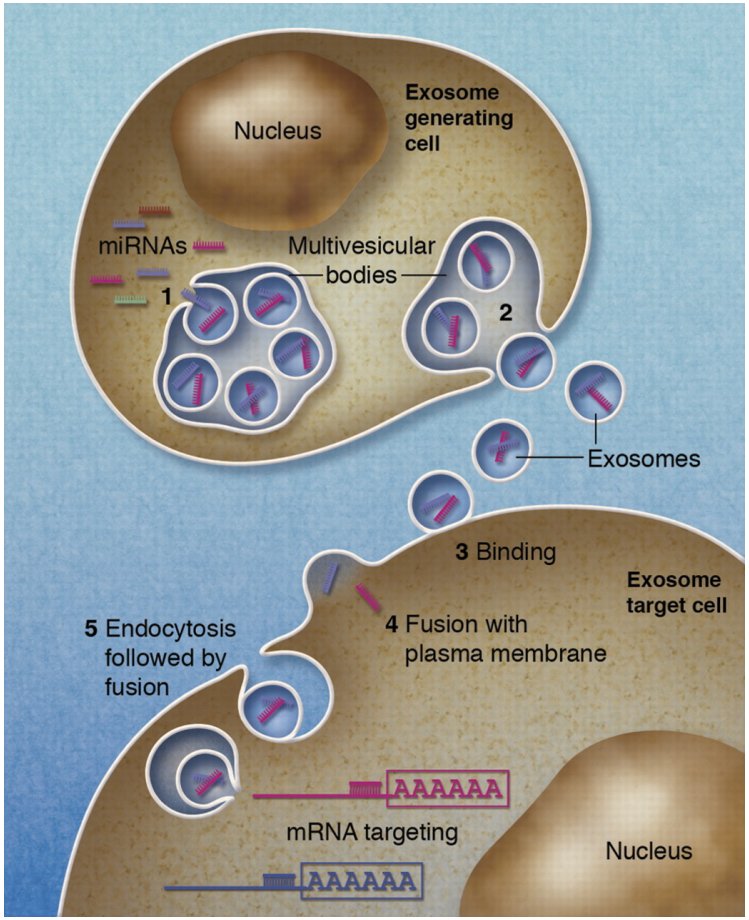


Photo: Stoorvogel W. Functional transfer of microRNA by exosomes. 2012. Blood: 119(3). DOI: 10.1182/blood-2011-11-389478

Why Use Exosomes?

- Secretion into body fluids[10]
  - Noninvasive testing
- Stability of contents
  - Long-term storage use[11]
- Better than current biomarkers[12]
- Diagnostics[10]
- Prognostics[10]
- Treatment[10]
  - Targeting exosome production
  - Targeting exosome contents
  - Monitoring treatment effectiveness

Our Goal:

- We want to find exosomal biomarkers for CVD in cases of metabolic syndrome (MetS) and diabetes
- This could help in diagnostics, prognostics, and even treatment of CVD
- Other exosome success stories:
  - Roles in cancer cell proliferation and metastasis[12,13]
  - Roles in tumor suppression[15]
  - Roles in kidney diseases, liver diseases, and neurological disorders[16,17,18,19,20]

Ossabaw Swine[21,22,23]

Active lean Inactive Obese

- Genetic predisposition to:
  - Obesity
  - Insulin resistance
  - Glucose intolerance
    - High (LDL/HDL)
  - Dyslipidemia
    - High (TG)
  - Hypertension
- Similar cardiovascular system to humans
  - Similar anatomy
  - Similar size
  - Similar pathology

Preparing the Pigs[24]

Control Pigs (2)

- Standard 2,400 kcal/day diet
  - 22% protein
  - 70% carbohydrate
  - 8% fat

MetS Pigs (1)

- High fat, 4,500 kcal/day diet
  - 13% protein
  - 40% carbohydrate
  - 47% fat

Treatment Group	Body Weight (kg)	Total Cholesterol (mg/dL)	Blood pressure (mm.Hg)
MetS	94	275	155/98
Lean	45.5	72	130.5/70

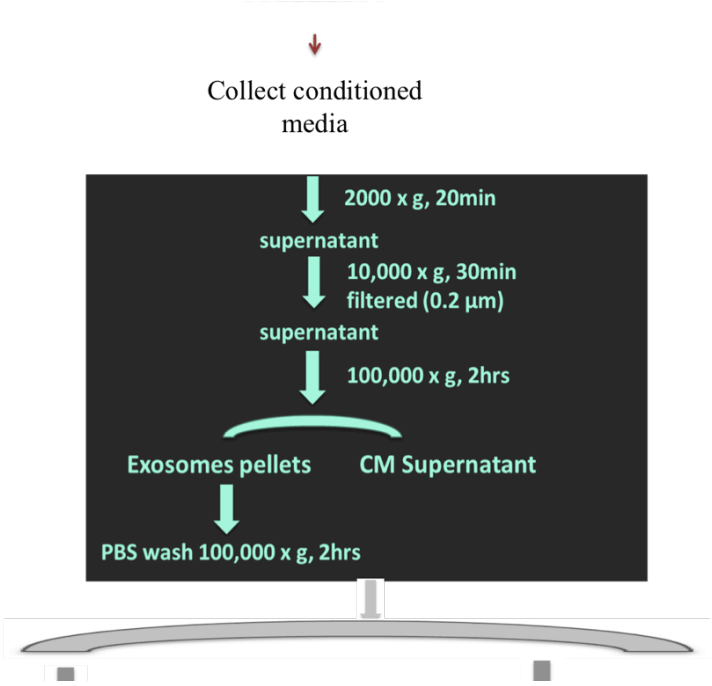
Both groups were treated for 11-12 months.

Isolation

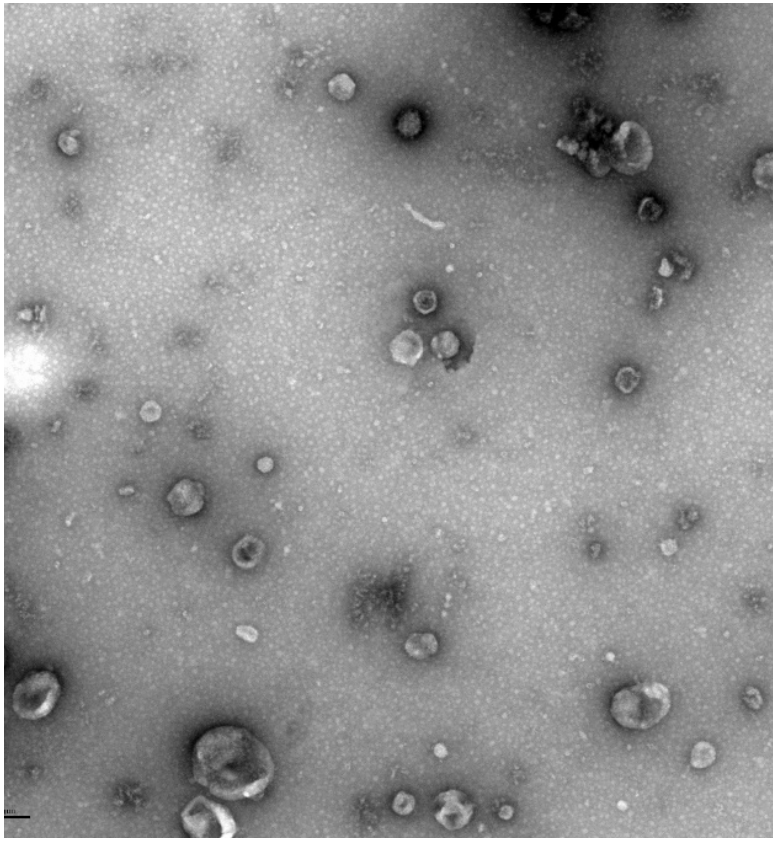
Isolating the Cells

- Left ventricles received were minced and rinsed with saline.
- Tissue was rinsed with collagenase.
- Selective attachment to tissue culture plastic was used – fibroblast cells were collected.
- This yields pure fibroblast culture.
- The cells were not divided; only primary cells were used.

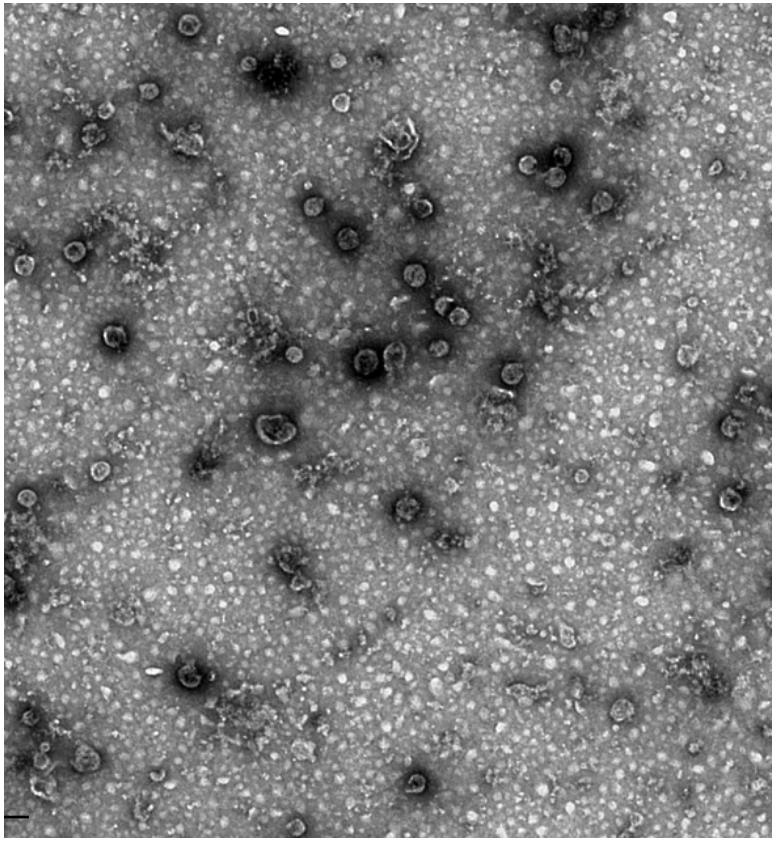
Isolating the Exosomes



Transmission Electron Microscopy

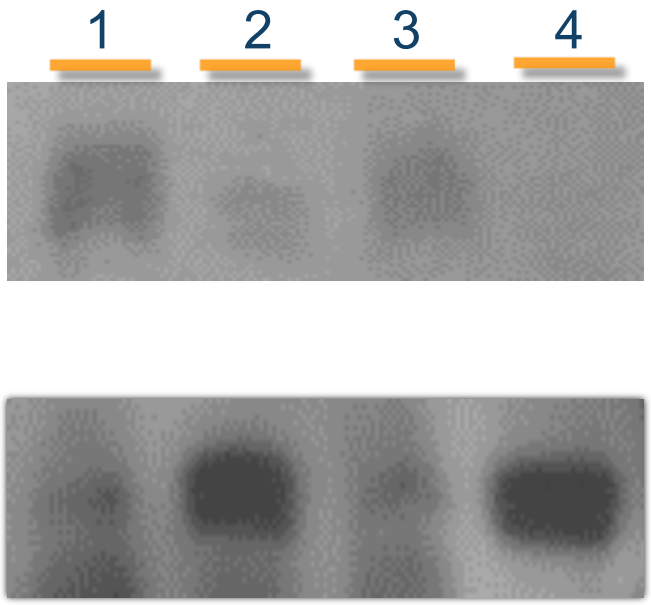


MetS



Control

Western Blot Analysis



1: Lean exosome
2: Lean cell
3. MetS exosome
4. MetS cell

Conclusion

- Exosomes were isolated and characterized from cardiac fibroblasts
- Future studies will involve analyzing the exosomal contents:
  - 2-D gel electrophoresis
  - Mass spectrometry
- A biomarker for CVD in metabolic syndrome, diabetes, and obesity could save thousands of lives in the most high-risk populations.

Sources

- 1.Heart Disease [Internet]. Atlanta (GA): Centers for Disease Control and Prevention: c2015 [cited 2015 April 9]. Available from: http://www.cdc.gov/heartdisease/index.htm.
- 2.The top 10 causes of death [Internet]. Geneva (Switzerland): World Health Organization. C2014 [cited 2015 April 9]. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/.
- 3. 2014 National Diabetes Statistics [Internet]. Atlanta (GA): Centers for Disease Control and Prevention: c2014 [cited 2015 April 9]. Available from: http://www.cdc.gov/diabetes/data/statistics/2014statisticsreport.html.
- 4. National Diabetes Education Program: The Link Between Diabetes and Cardiovascular Disease [Internet]. Wasington D.C. (USA): US Department of Health and Human Services. c2007 [cited 2015 April 9]. Available from: http://ndep.nih.gov/media/CVD_FactSheet.pdf.
- 5. Overweight and Obesity [Internet]. Atlanta (GA): Centers for Disease Control and Prevention: c2013-2014 [cited 2015 April 9]. Available from: http://www.cdc.gov/obesity/data/prevalence-maps.html.
- 6. O'Sullivan AK, Rubin J, Nyambose J, Kuznik A, Cohen DJ, Thomson D. 2011. Cost Estimation of Cardiovascular Disease Events in the US. Pharmacoeconomics, 29(8): 693-704

Sources

- 7. Jaspers L, Colpani V, Chaker L, van der Lee SJ, Muka T, Imo D, Mendis S, Chowdhury R, Bramer WM, Falla A, Pazoki R, Franco OH. 2014. The global impact of non-communicable diseases on households and impoverishment: a systematic review. European Journal of Epidemiology. ISSN 1573-4284.
- 8. The Cost of Diabetes [Internet]. Alexandria (VA): American Diabetes Association: C2013-2014 [cited 2015 April 9]. Accessed March 2, 2015. Available from: http://www.diabetes.org/advocacy/news-events/cost-of-diabetes.html.
- 9. Stoorvogel W. Functional transfer of microRNA by exosomes. 2012. Blood: 119(3). DOI: 10.1182/blood-2011-11-389478.
- 10. Kawikova I and Askenase PW. Diagnostic and therapeutic potentials of exosomes in CNS diseases. Brain Research (2014). DOI: 10.1016/j.brainres.2014.09.070
- 11. Ge Q, Zhou Y, Bai Y, Xie X and Lu Zuhong. miRNA in Plasma Exosome is Stable under Different Storage Conditions. 2014. Molecules. 19: 1568-1575. DOI: 10.3390/molecules19021568
- 12. Müller G. Microvesicles/exosomes as potential novel biomarkers of metabolic diseases. 2012. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2012:5. 247-282. DOI: 10.2147/DMSO.S32923

Sources

- 13. Tickner JA, Urquhart AJ, Stephenson S-A, Richard DJ and O'Byrne KJ. Functions and therapeutic roles of exosomes in cancer. 2014. Frontiers in Oncology. 4(127). DOI: 10.3389/fonc.2014.00127
- 14. Mishra PJ. Non-coding RNAs as clinical biomarkers for cancer diagnosis and prognosis. 2014. Expert Rev Mol Diagn. 14(8): 917-919.
- 15. Tickner JA, Urquhart AJ, Stephenson S-A, Richard DJ and O'Byrne KJ. Functions and therapeutic roles of exosomes in cancer. 2014. Frontiers in Oncology. 4(127). DOI: 10.3389/fonc.2014.00127
- 16. Salih M, Zietse R and Hoorn EJ. Urinary extracellular vesicles and the kidney: biomarkers and beyond. 2014. Am J Physiol Renal Physiol. DOI: 10.1152/ajprenal.00128.2014
- 17. Bala S, Petrasek J, Mundkur S, Catalano D, Levin I, Ward J, Alao H, Kodys K, and Szabo G. Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced and inflammatory liver diseases. 2012. Hepatology. 56(5): 1946-1957.
- 18. Rodríguez-Suárez E, Gonzalez E, Hughes C, Conde-Vancells J, Rudella A, Royo F, Palomo L, Elortza F, Lu SC, Mato JM, Vissers JPC and Falcón-Pérez JM. Quantitative proteomic analysis of hepatocyte-secreted extracellular vesicles reveals candidate markers for liver toxicity. 2014. Journal of Proteomics. 103(2014): 227-240.

Sources

- 19. Saenz-Cuesta M, Osorio-Querejeta I and Otaegui D. Extracellular Vesicles in Multiple Sclerosis: What are They Telling Us? 2014. Frontiers in Cellular Neuroscience. DOI: 10.3389/fncel.2014.00100
- 20. Banigan MG, Kao PF, Kozubek JA, Winslow AR, Medina J, Costa J, Schmitt A, Schneider A, Cabral H, Cagsal-Getkin O, Vanderburg CR and Delalle I. Differential Expression of Exosoml microRNAs in Prefrontal Cortices if Schizophrenia and Bipolar Disorder Patients. 2013. 8(1): e48814.
- 21. Sturek et al. In *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*, 2nd Ed. M. Swindle (Ed.). p. 397, 2007
- 22. Lee et al. *Hepatology* 50:56, 2009
- 23. Edwards, Neeb et al. *Cardiovasc. Res.* 85:631, 2010
- 24. Sham JG, Simianu VV, Wright AS, Stewart SD, Alloosh M, Sturek M, Cummings DE, and Flum DR. Evaluating the Mechanisms of Improved Glucose Homeostasis after Bariatric Surgery in Ossabaw Miniature Swine. 2014. Journal of Diabetes Research, 2014 (2014). DOI: 10.1155/2014/526972

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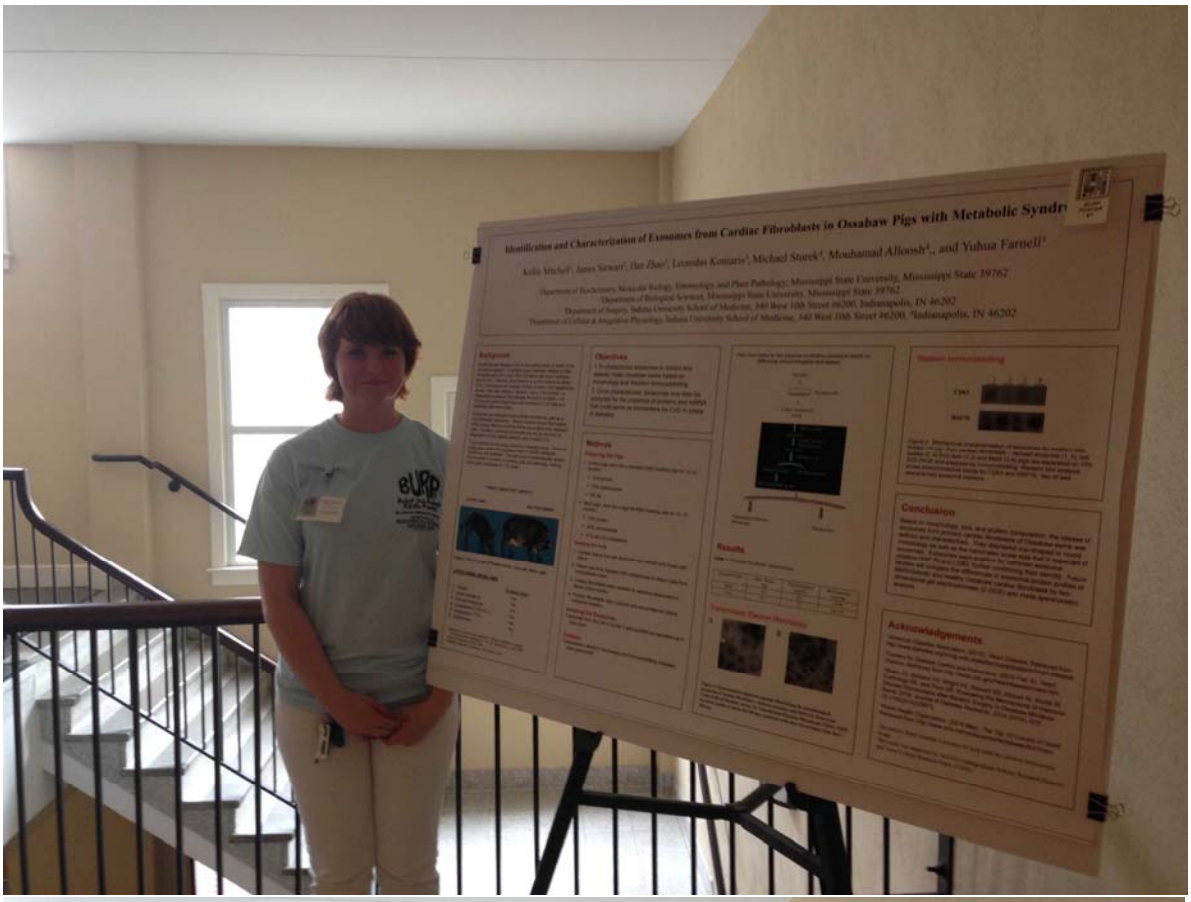
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Conclusion
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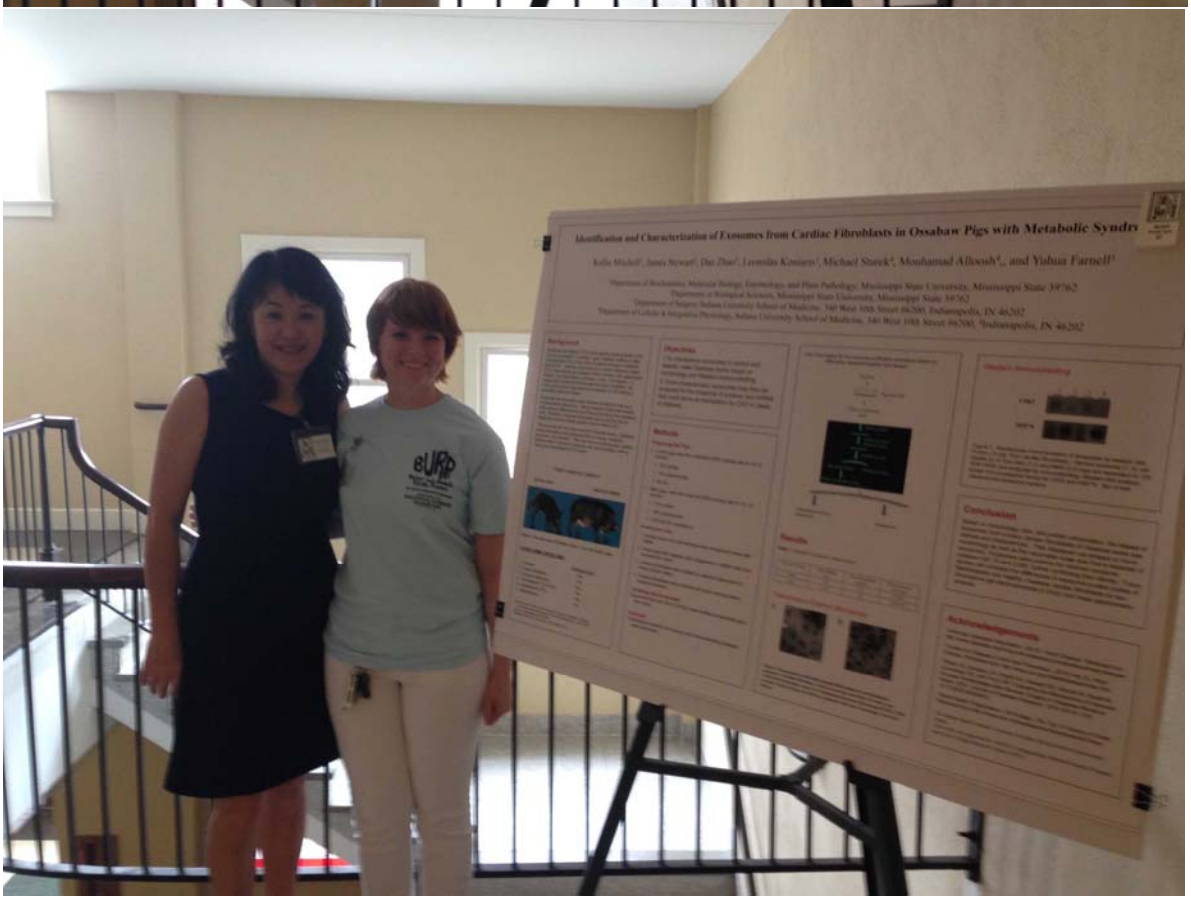
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