Understanding metabolic diseases

Professor Eugénia Carvalho outlines her research activities, which are geared towards understanding the molecular mechanisms of insulin resistance and wound healing in diabetes

AT the University of Coimbra, Portugal, Professor Eugénia Carvalho and her team at the Center for Neurosciences and Cell Biology are interested in understanding the molecular mechanisms of insulin resistance and wound healing in diabetes. Primarily, their work is designed to address questions in order to evaluate alterations in insulin action and intracellular signalling in post-transplant diabetes and cardiovascular disease (CVD) by understanding the role of the adipocyte as an endocrine organ. Here, Carvalho tells *SEQ* about their work in these areas and where her future research interests may lie.

Is enough attention being paid to the role that epicardial adipose tissue (EAT) plays as an important factor in the pathogenesis of metabolic-related cardiac diseases? Can you describe some of your own work in this area?

Epicardial fat is a very different fat depot compared to the fat depots that people have been studying for years. Unlike other depots, epicardial fat is hardly present in rodents, and therefore many of those who are studying fat metabolism do not get to it unless they study higher mammals, vertebrates, which contain this kind of fat, particularly humans.

Epicardial fat surrounds the heart and is present in very specific areas. Everyone has it, regardless of whether they are healthy or whether they have a pathology. It is there for a reason, but that reason is as yet poorly understood. The functional and anatomic proximity of EAT to the myocardium enables endocrine, paracrine, and vasocrine effects upon the neighbouring myocardium.[1]

What we do know is that any fat depot that is dysfunctional will respond in a dysfunctional manner, and epicardial fat is a tissue which plays a very important role in cross-talking with other tissues that are metabolically active. If this fat cell isn't working properly, it may pass the wrong message across to the working myocardium. Epicardial fat displays a distinct and large secretome which regulates physiological and pathophysiological processes in the heart.

We have collected over 200 paired EAT and subcutaneous (SAT) fat biopsies from people undergoing open heart surgery and have evaluated insulin-stimulated glucose uptake and lipolysis and found these processes to be very distinct on the isolated cells from these two fat depots, in paired samples from the same subjects.[2] These fat biopsies were obtained from individuals with and without diabetes but with heart failure. After comparing these isolated cells, we discovered that EAT cells are smaller in size than the SAT cells are.[2] EAT cells are more similar to the brown fat cell, in that they express more uncoupling protein (UCP)1 than SAT cells do. They have a different phenotype and therefore they need to be better understood from the molecular and physiological points of view in order to better assess their prominent role in the physiological and metabolic processes particularly involving the neighbouring myocardium and vasculature, and why they are there in the first place.

Besides glucose and lipid metabolism we are in the process of investigating EAT's mitochondrial oxidative capacity and endoplasmic reticulum stress levels.[3] Because the heart is a pumping muscle, it is working all the time as opposed to skeletal muscle, it needs fuel constantly whether we are sleeping or awake, and it is the EAT cell, at least in part, that provides with the fuels in the form of lipids and other metabolites, including adipokines and other immune-modulating factors into the surrounding area. Thus, it is so important to understand the physiology of this fat depot in relation to CVD and heart failure.

miRNAs are involved in a number of biological processes, including the pathogenesis of disease. How would you like to see them being better explored when it comes to attempting to understand the molecular mechanisms underlying chronic complications in diabetes?

miRNAs are extremely important regulatory molecules, but they have only recently become a focus for research regarding the complications of diabetes.[4]

We know that fat cells secrete exosomes which contain proteins, RNAs, and microRNAs (miRNAs), molecules that are secreted into the circulation where they will act as important signalling molecules, triggering cellular activation, as well as immune-modulatory effects on other nearby tissues. These processes are, however, not very well understood, and there is still a lot of contradicting results regarding their modes of action.

It is therefore important to understand, for example in relation to cardiac complications, the role of epicardial fat and its miRNA secretion profile, and whether there are specific miRNA signatures in EAT which could inform on novel biomarkers for heart disease treatments.

On the other hand, and in regard to the microvascular complications of diabetes, specifically diabetic foot ulceration, we have recently conducted a large miRNA screening on diabetic mouse skin *versus* non-diabetic skin, in the presence or absence of wounds. From this study, we were able to observe a large difference

in miRNA expression levels, when comparing healthy *versus* diabetic conditions.[5]

We have chosen several of these miRNAs that were elevated under diabetic conditions and we are now using miRNA inhibitors to target and reduce their expression at the skin level. When we decrease the levels of miRNAs that were found elevated under diabetic conditions, we observed that inflammation decreases and wounds heal better if these miRNAs have been inhibited. I think there is the potential here for finding very specific and early biomarkers that may be able to inform at an early stage about diabetic ulcer development, much before the ulcer is even present.

Conversely, if certain miRNA levels are already too low in the skin under diabetes, leading to impaired wound healing, then their expression will need to be increased until tissue regeneration and healing is normal. This could be done by using miRNA mimics that would target the decreased miRNAs locally at the wound site. As such, we have conducted two studies simultaneously which are looking at both sides of this with regard to very specific miRNAs in question, because we can use them as therapeutic signatures and, eventually, they could be utilised for topical applications instead of systemic treatments, which may be dangerous in other places in the body.

For example, clinicians may not be able to completely heal the pathology of a diabetic foot – where many times the major problem is bacterial infection – just by using systemic antibiotic treatments. In cases where patients have neuropathy, antibiotics may very well not be delivered to the wound site due to poor blood flow at the wounds site. Antibiotics or other medications will only get to the extremities partially, if at all. This may mean that the bacteria present are likely going to stay there and, what is more, they will in all likelihood be developing resistance to those antibiotics. This could be addressed by administering the antibiotics topically rather than systemically, for instance.

We also know that certain peptides can kill bacteria – antimicrobial peptides (AMPs) are found, for example, in tears and saliva, as the eyes and mouth are the two major areas which are open to microbes all of the time. However, we also know that some people with diabetes have lower levels of these antimicrobial peptides, and so if they are made specific to the bacterial tissue and administered to the wound site, bacteria will be targeted, and the wound will heal better.

We have therefore begun a project that is working to define the different bacterial population at the wound site in diabetics *versus* non-diabetic patients in an effort to identify the specific bacterial strains that maybe causing infection and inflammation at the wound site.[6]

To do this, we are attempting to construct antimicrobial peptides guided specifically to those bacterial types in order to kill them and perhaps disturb and disrupt the biofilm that may have been formed and which is not going to be affected by antibiotics.

We hope, moving forwards, to be able to combine the two therapies into one application and apply them topically to the wound, thereby decreasing infection and inflammation at the same time.

What have been your biggest achievements thus far when researching the area of diabetic wounds?

We started working on wound healing in diabetes a few years ago and some of the work was performed in collaboration with colleagues at Harvard. Here I set up the wound healing model in rodents. I had initially worked with Substance P and obesity and one day we decided to apply Substance P to a diabetic mouse wound.[7] Our hypothesis was right.

Because many diabetics have neuropathy and therefore poor blood flow and poor oxygenation, diabetic foot wounds do not receive oxygen and other healing peptides, including Substance P and immune-modulators that are needed for normal healing when we get injured; thus, under diabetes conditions wound healing is delayed and does not follow the same healing processes one would expect therefore many patients develop chronic non-healing foot ulcers.

We have since then conducted numerous studies in the presence and the absence of diabetes where we have shown that if certain neuropeptides, including Neurotensin and Substance P are applied topically to a skin wound under diabetes conditions the wound will heal much faster.[7-9]

Moving forwards, where will your research priorities lie?

I will continue working on cardiac adipose tissue, epicardial fat, as well as on the role of miRNAs and AMPs for topical diabetic foot ulcer treatment. One other very important topic that has been somewhat neglected by the research community is drug-induced metabolic dysfunction, one of the topics of one of our European research consortia.

Nowadays, especially in the USA but also in Europe, various medications are prescribed even to young children, sometimes with no real need. Many of these medications can cause metabolic dysfunction including diabetes as a side effect, and yet those taking them need to continue to do so for the rest of their lives, meaning that they stand to become diabetic.

It is not yet known how some of these drugs actually work in terms of their molecular pathways, and therefore we have done studies where we treated cells with immunosuppressive agents, including cyclosporine and rapamycin, in collaboration with colleagues in Sweden, so as to better understand some of the mechanisms underlying their modes of action. These drugs may act via different pathways but both cause insulin resistance *in vivo*.[10-12]

Drug-induced metabolic diseases are understudied studied and much more needs to be done so that new drugs can be developed to replace the older ones so that these side effects are avoided.

We have just recently started a European consortium funded by Horizon 2020 to precisely evaluate some of the metabolic effects of some of the second generation anti-psychotics that can cause severe metabolic dysfunction.

References

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