To begin, could you give an overview of your current role and academic background?

I am tenured Associate Professor of Pathology and Associate Research Professor of Neurosurgery at the University of Michigan Medical School. I am actively involved in two graduate programmes: Molecular and Cellular Pathology, and Neuroscience. My primary research is focused on identifying the molecular mechanisms of cerebrovascular diseases with special emphasis on the mechanisms underlying blood-brain barrier (BBB) dysfunction in pathologies like stroke, cerebral cavernous malformation (CCM) and neuroinflammation. My early work was mostly directed towards investigating how inflammatory mediators such as cytokines and chemokines remodel the cell-cell junctional complex and facilitate inflammatory cell migration in the brain during the progression of inflammation.

What is your primary research objective, and how have your previous investigations informed your present focus?

My goal is to identify and understand the remodelling of the brain endothelial junction complex during and after BBB hyperpermeability. For several years, my research was focused on the inflammatory remodelling of the tight junction complex during acute and subacute inflammation after stroke. Inflammation is one of the most common responses to the different pathological stimuli in the brain, and it is in the background for developing BBB hyperpermeability in various pathological conditions.

CCMs are the most common brain vascular malformations, causing patients to present with seizures, focal deficits, nonspecific headaches or haemorrhagic stroke. How do you intend to better understand the BBB impairment in CCMs and how it contributes to disease pathogenesis?

CCMs are considered the only brain vascular malformation in which the pathological substrate is associated with alterations in the microvasculature and the BBB. CCM lesions have poorly formed brain-endothelial barriers, with gaps often noted between individual cells, resulting in increased permeability of the BBB by MRI. One unresolved issue here is whether BBB impairment occurs as a primary process, causing unstable and leaky vessels that lead to the inflammatory response, or is instead a consequence of intensive inflammatory response developed due to the accumulation of unwanted blood compounds. Over the last three years, my group has been investigating how impairment of the BBB occurs in the development of a CCM lesion, specifically focusing on the tight junctional complex.

What have been the most prominent challenges you have encountered in your research to date, and how have you overcome them?

One of the biggest challenges in my work is to develop a good animal model to research the cerebral cavernous malformations 3 (CCM3). There are several laboratories that are trying to do this; it’s a very important issue due to the fact that genetically engineered knockout mice are embryonically lethal, and earlier attempts to appropriately genetically engineer mice have resulted in inadequate lesion development. Adequate animal models would allow us to follow the lesion in real time and test different targets for potential therapy. I think that we are very close to resolving this problem.

Do you have plans for further research in this field?

My colleagues in this field and I are just starting to understand the intricate structure and function of the tight junction complex at the BBB, and its role in vascular hyperpermeability. My plans regarding this project are to identify good signalling targets that could improve the altered cortactin function in CCM3 lesions and improve the stability of the tight junction complex. We have some candidates for this function, including a number of signalling molecules, but firm answers are a very long way ahead. I would like to find potential pharmacological treatment for CCM3 and similar CCM lesions, as neurosurgical procedures are sometimes very difficult. One promising approach in this direction would be to utilise the inhibitor of Rho kinase (ROCK) for the treatment of CCM2 lesions, which is now at the clinical trial stage.
Junction integrity

A group of scientists at the University of Michigan Medical School, USA, has been working to improve current understanding of tight junction complexes, with a view to enabling new therapeutic and drug-delivery options

THE BLOOD-BRAIN BARRIER (BBB) is one of the miracles of the human anatomy; through the judicious application of impermeable connections known as tight junctions between cells, the body creates a highly selective barrier between the blood and the central nervous system that is extremely efficient at excluding unwanted elements.

This barrier is such a dedicated guardian of health, in fact, that it even refuses entry to drugs that target the brain – and this is where problems arise. It is a cruel irony that a barrier sculpted by evolution to safeguard human health should ultimately hinder it, but the BBB has nonetheless proven a colossal obstacle to scientists and medical professionals hoping to treat problems in the brain with drugs rather than surgery. What is more, the mechanisms by which it refuses these treatments are still not fully understood.

CELLULAR SOLIDARITY

The tight junction complex that exists between endothelial cells is the key to the BBB’s impermeability. Such solidarity between cells is achieved through a multitude of constituent parts, but might be readily visualised as two cell membranes stitched together with threads of protein complex, leaving no room between them for molecules to pass through. The impassable connection therefore forces molecules to enter the brain through endothelial cells, which are able to strictly regulate which elements are admissible and which are not. The tight junctions are also important because they hold all endothelial cells in place, ensuring that the polarity of cells is fixed. Without this guarantee, the selective permeability of the barrier could not be maintained.

CAVERNOUS CATASTROPHES

Cerebral cavernous malformations (CCMs) are the most common malformation of the brain’s vascular system, and although a quarter of sufferers never experience any symptoms, those who do are vulnerable to seizures, paralysis, cerebral haemorrhage and even death. This threat alone is enough to draw the attention of medical researchers – but it is also possible that understanding the breakdown of the BBB represented by CCM cases could shed light on the workings of this mysterious barricade.

One team at the University of Michigan Medical School, USA, is examining this issue as part of a research programme aimed at understanding the exact nature of the tight cell junction in brain endothelial cells. It is now known that around 50 different proteins make up its dynamic structure, and recent years have seen researchers begin to identify some of the signalling networks and structural characteristics responsible for the efficacy of the junction – but there is much to discover in this area yet. Led by Associate Professor of Pathology and Associate Research Professor of Neurosurgery, Dr Anuska Andjelkovic-Zochowska, the Michigan lab hopes to make advances in understanding that could be translated into novel ways to manipulate the BBB, both to prevent leakage in disease pathology and to increase permeability selectively for drug delivery.

A NEW STUDY

In the last few years, scientists have identified the genes responsible for inherited or familial CCM, as well as the proteins CCM1, CCM2 and CCM3 that, through their absence, appear to cause a malformation in the affected genotypes. However, the pathogenic mechanisms responsible for hyperpermeability on a cellular level are still not well understood. That is why Andjelkovic-Zochowska’s lab has recently engaged in a new project designed to elucidate critical molecular events in the maintenance of BBB integrity, and how these are altered in cases of CCM3 absence. The study will aim to evaluate the functional and morphological consequences of CCM3 loss on the tight junction complex’s interaction with the actin cytoskeleton, as well as the interactions between various components of the tight junction complex.

The methodology employed in the project involves analysing BBB integrity in a CCM3 lesion in two ways: firstly, using microarray and proteomic analysis of tight junctional proteins in microvessels collected from patients with diagnosed CCM3, and secondly, by investigating an in vitro model of CCM3 developed by depleting its expression in a sample of human brain endothelial cells. In order to define the protein-protein interactions within CCM3-mutated complexes, which are important to barrier integrity, the researchers used various cellular and molecular biology methods including fluorescence resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) analysis, and subsequent analysis of the proteins’ phosphorylation status. Alongside these approaches, a functional assay for brain endothelial barrier integrity was performed, evaluating permeability. More recently, the group has begun attempting to develop animal models.

GOING FORWARD

Although the project is not yet complete, Andjelkovic-Zochowska and her collaborators have already produced some interesting results. Concurrent with previous studies, the first screen
THE MECHANISM OF BLOOD BRAIN BARRIER IMPAIRMENT IN CEREBRAL CAVERNOUS MALFORMATIONS

OBJECTIVES
• To investigate the underlying mechanism of blood brain barrier (BBB) impairment in cerebral cavernous malformations (CCMs), focusing specifically on the critical property of the BBB endothelial cell-cell junctional complex known as the tight junction
• To develop novel therapeutic strategies to restore vascular hyperpermeability

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Amy L Akers, Angioma Alliance, USA

FUNDING
National Institutes of Health (NIH)

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Disease and discovery
In their efforts to understand the BBB, Andjelkovic-Zochowska and her collaborators have focused on CCMs – but this is just one group of conditions that involves BBB dysfunction. The team has also been responsible for investigating a number of other conditions that involve barrier dysfunction, and their results may have useful applications for the treatment of these conditions.

CHRONIC STROKE
In chronic stroke cases, a loss of vascular structural integrity and prolonged ischaemia can lead to breakdown of the BBB, which often contributes to secondary progressions of brain injury; cerebral oedema – the accumulation of water in extracellular spaces within the brain – is an example of this.

AGEING
Ageing is associated with a number of changes in BBB function that, in combination with vascular changes such as hypertension and ischaemia, can lead to neurological disorders such as dementia and Alzheimer’s disease. Because these changes in circulation are also associated with ageing, preserving the BBB in the older population is extremely important.

DIABETES
In diabetes mellitus, microangiopathy is known to lead to complications including blindness, kidney failure and peripheral neuropathy, but recent clinical studies have suggested that the BBB changes induced by the disease can cause vascular dementia, haemorrhages and even lead to Alzheimer’s disease.

analysis of the tight junction complex in cells collected from patients showed that changes in how the proteins were expressed were minimal. However, the analysis of interactions between proteins revealed diminished relationships between some constituent proteins, and in particular between proteins and the actin cytoskeleton. This is important because the tight junction is not just a structural bond, but a dynamic one, meaning that protein-protein interactions could potentially change its behaviour. One protein that had significantly reduced expression and function in CCM3 cells was cortactin, the bridge protein that connects structural protein ZO-1 to the actin cytoskeleton. The researchers’ hypothesis is that this is the change in CCM3 cases that renders the barrier unstable.

Looking ahead, the Michigan team plans to further develop understanding of this interaction. They are hopeful that additional information on the function of CCM3 will not only give a greater insight into a potentially lethal malformation and its possible therapies, but also aid understanding of the BBB.

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