An ageing paradigm

Dr Vered Raz is investigating how the RNA landscape changes during muscle ageing. She discusses the ways in which RNA processing impacts disused and damaged muscles, and her efforts to understand the dynamics and complexity of ageing processes in humans.

Why have you chosen muscle as the tissue in which to focus your studies on ageing?

My research focuses on skeletal muscles since they compose the majority of our body mass, and have been shown to correlate with health and predict longevity in older people. There are well-established methods for working with this tissue; late-onset muscle disorders are often monogenetic (thus testable in model systems) and it is feasible to obtain muscle samples for ex vivo and in vitro studies, as well as measuring changes in muscle topology using magnetic resonance imaging. In humans, there are about 400 different skeletal muscles, and their ageing-associated tissue degeneration is highly varied. Unravelling molecular differences between muscles creates a novel opportunity to identify the factors that make a tissue resistant to ageing.

Can you elaborate on the experimental approach you take to your research?

To study molecular ageing, I employ two experimental models to generate ribonucleic acid (RNA) expression datasets: affected muscles from the late-onset muscle disorder oculopharyngeal muscle dystrophy (OPMD) and muscles from otherwise healthy subjects. From the muscle samples, we generate RNA expression profiles and investigate candidates, in particular those related to RNA processing and protein catabolism. With molecular genetic tools we investigate a functional role using cellular and animal models. Then, we probe the potential of small molecules to restore poly(A) binding protein 1 (PABPN1) expression level and phenotypes.

What is OPMD and why is it often underdiagnosed?

OPMD is a late-onset autosomal dominant muscular disorder. The disease is rare, affecting one in 100,000 individuals in Western countries, yet is possibly underdiagnosed due to mild symptoms and late onset. Because of the limited number of patients per nation, research into this disease should be conducted via an international consortium.

At present, treatment options for patients are limited. Surgeries for degenerative eyelid and pharyngeal muscles – the first symptoms of the disease – are only a temporary solution, as the basic defects in affected muscles are not corrected and thus muscle weakness often reappears after just a few years. Eating difficulties and reduced mobility in OPMD patients are often associated with social restrictions and limited independence. Although the muscle weakness is often not fatal, symptoms severely affect quality of life. I propose that muscle weakness in OPMD can be used as a model for accelerated muscle ageing. Thus, research on OPMD could be beneficial for understanding both patients and muscle disuse in the elderly population.

Could you highlight some of the biggest challenges you have faced in this investigation? How were you able to overcome these?

Studying a rare disorder is a big challenge, as patient samples are limited. Thus, I have launched many national and international collaborations to overcome this problem. This showed me the diversity of symptoms, as well as genetic, social and environmental confounders, which are all poorly understood at present. It is also a challenge to demonstrate that a monogenic heritable muscular disorder could represent a physiological process, such as ageing. Revealing molecular similarities could advance understanding of both molecular processes associated with/leading to OPMD and physiological ageing. Identifying potential molecules for drug development is an additional challenge, which can be only overcome through creating a multidisciplinary research team and international collaborations.

Are there any key achievements from your career to date of which you are most proud?

My main achievement is the classification of OPMD as a muscle ageing disorder, representing accelerated ageing, and the discovery that PABPN1 is a limited factor in OPMD and ageing muscles. The latter suggests that loss of PABPN1 function, rather than toxicity, leads to muscle weakness in OPMD and older people. Moreover, this raises a novel role for RNA processing in ageing.

To what extent is a multidisciplinary approach important for your work?

Uncovering complex, multifactorial processes – in which time plays a central role and many molecular processes are impaired – can only be achieved by combining multiple approaches. Although my research experience is highly diverse – spanning genetics, molecular biology, biochemistry, bioinformatics, biophysics and molecular medicine – it is the multiple and diverse collaborations with clinicians, physicists, chemists, statisticians and computer scientists that allow me to address novel questions in this complex area.
Maintaining the RNA landscape

Geneticists at Leiden University Medical Center, Netherlands, are studying molecular ageing in human tissues. Their multidisciplinary investigations of RNA expression in skeletal muscle could generate new treatments for muscle degeneration.

HUMAN AGEING IS characterised by the progressive loss of control in multiple cellular and metabolic processes, leading to impairment of muscle degeneration, which together increase the risk of diseases such as cancer, diabetes and neuromuscular disorders. Of these, loss of muscle strength and mass starts after the age of 50 and is particularly prominent in older individuals.

In late-onset neuromuscular disorders, muscle loss is more severe, leading to degenerated muscles. Therefore those conditions can be considered as accelerated muscle ageing. As human life expectancy grows, the prevalence of muscle weakness in disease conditions rises, leading to declining mobility and stability – a pattern that is associated with a huge social and economic burden. Dr Vered Raz is one prominent researcher who is working to understand the molecular basis of ageing in muscle, which will enable the development of novel treatments to lessen this burden. She leads a research group in the Department of Human Genetics at Leiden University Medical Center that is conducting multidisciplinary research to decipher age-associated muscle function decline.

PATHOGENIC MUSCLE DEGENERATION

In order to study muscular ageing, Raz is using oculopharyngeal muscular dystrophy (OPMD) as a disease model. This heritable disorder, which tends to emerge in the fifth decade of life, leads to progressive muscle degeneration and is an archetypal example of a late-onset neuromuscular protein aggregation disease.

OPMD initially manifests as a drooping of the eyelids due to weakness of the eye muscles, and swallowing difficulties due to weakness of the pharyngeal muscles. In addition, proximal limb weakness develops, usually affecting the muscles in the upper legs and hips. As the disease progresses, additional muscles are affected, resulting in severe difficulties with mobility, eating and, consequently, socialising and normal functioning. Since similar muscle weakness can occur as a part of natural ageing, it has been suggested that the disease is underdiagnosed.

At present, the only available medical option for OPMD involves invasive surgery to remove degenerated eyelid muscles and pharyngeal muscles. As the procedure does not repair the molecular damage, this is only a temporary solution. Clearly, this mild but devastating disease, which can impede mobility, independence and quality of life, demands further investigation. Furthermore, because it represents a model of accelerated muscle ageing, more knowledge about this disease could yield insights that apply to ageing conditions more broadly, particularly the general muscle decline associated with increasing longevity.

MOLECULAR INSIGHTS

While research of this nature is clearly needed, it is also extremely challenging, owing to the complex multifactorial nature of human ageing. Scientists still understand very little about the temporal and spatial changes associated with growing old, while longitudinal studies, which follow a subject over time and promise to provide meaningful molecular insights, are often not applicable. Raz is therefore extracting information from cross-sectional datasets – that is, datasets collected at one point in time from many different subjects.

In order to mine the molecular signatures of ageing, Raz and her team analyse RNA expression profiles in muscle that change during the ageing process. The researchers recently applied this methodology to several RNA expression studies from various tissues revealing temporal changes of RNA deregulation between tissues. This study points to the molecular processes that are initially affected during ageing, which could prove effective targets for interventions or therapies that delay ageing.

MUSCLE CHANGES

RNA expression analysis has allowed Raz to identify poly(A) binding protein 1 (PABPN1) as a limiting factor in OPMD and likely also in normal muscle ageing. PABPN1, a multifunctional regulator of RNA processing,
Therapeutic Developments

Raz’s research has important potential clinical applications. For instance, in both OPMD and normal ageing, low levels of PABPN1 lead to the utilisation of alternative polyadenylation sites (APA), in turn triggering a global change in the RNA landscape and expression profiles. Because PABPN1 blocks APA site utilisation, this protein could prove to be a useful therapeutic tool for delaying the onset of OPMD and normal muscle degeneration. Excitingly, Raz’s team has found that masking the APA of regulatory genes via AON technology restores PABPN1 levels and reverts myogenic defects, which could be a highly novel therapeutic option in the future.

Dr Vered Raz is one prominent researcher who is working to understand the molecular basis of ageing in muscle, which will enable the development of novel treatments to lessen this burden.

The expression of the gene encoding PABPN1 also changes with age and correlates with muscle symptoms in OPMD. Through comparing genome-wide RNA expression profiles from carriers of the mutant gene with healthy controls, Raz found that from midlife onwards, the level of PABPN1 mRNA expression in skeletal muscle progressively declines. This reduction also causes defects in muscle cell biology and muscle atrophy. An exciting finding, this could pave the way for sorely needed new treatments for the condition. “If we can restore the level of PABPN1, without causing its aggregation, we could delay muscle weakness in older people and OPMD,” Raz explains.

While OPMD is generally classified as muscular dystrophy, Raz’s RNA expression studies have actually shown more similarities with normally aged muscle, revealing similar molecular signatures in muscles from elderly individuals. Her findings could therefore also be relevant for understanding the mechanisms underlying healthy ageing. Indeed, OPMD’s late-onset, age-associated progression, and its similar symptoms to the ageing process more generally, suggest that OPMD represents an accelerated form of normal muscle ageing. This finding has been corroborated by studies showing that molecular pathways deregulated in OPMD are also affected in normal ageing; moreover, a faster change in expression level for a gene cluster including PABPN1 was found in OPMD compared with normal ageing. Therefore, OPMD could also improve understanding of sarcopenia, the normal degenerative loss of skeletal muscle in the older people, and perhaps suggest a means of slowing or even delaying this pathology.

Age Positions

More recently, Raz has extended her efforts to investigate ageing-associated RNA expression changes elsewhere in the body. Alongside experts in medical statistics and computer science, she has studied six cross-sectional RNA expression profiles in a range of human tissues in order to identify the age-related differences. This revealed key changes at two distinct age positions in muscles in the quadriceps and brain cortex. In the skeletal muscle, the first age position was at 40 years, whereas in the brain it was around 50, suggesting that expression changes in skeletal muscles occur earlier than in the brain. The second age position was the same in both datasets, at around 70 years. Functional mapping of the genes at each position indicated that calcium homeostasis and lipid metabolism are affected first, while apoptosis and hormonal signalling pathways become deregulated at the second time point.

Taken together, these results indicate that age-associated temporal changes in human tissues progress through distinct ‘age positions’ that differ molecularly between tissues. This study provides the quantitative description of the temporal changes of ageing that has, to date, been so elusive.