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Epigenetics and the quest for personalised CNS drugs

The progressive understanding of tumour cell biology has revolutionised the treatment of cancer in recent decades. By categorising malignancies into subclasses with common molecular features, we are now able to produce personalised therapies that co-exist with generic chemotherapies. Their use is directed by an understanding of the genetic susceptibility of a patient and/or genetic and epigenetic changes in his or her tumour. A growing number of malignancies that used to be lethal have finally seen a dramatic improvement in their prognosis.

Personalised therapies may also have a role in treating diseases of the central nervous system (CNS). The path to achieving this goal may well come through epigenetics.

In this article, I discuss how epigenetic mechanisms are driving diseases of the CNS and explain what our company, Oryzon Genomics SA, is doing to find new treatments. The focus of the article is on lysine-specific demethylase 1 (LSD1), a specific epigenetic enzyme.

Epigenetics in CNS disease

According to the US National Institutes of Health, epigenetics is an emerging field of science that focuses on the study of changes in gene function that are heritable but not attributed to alterations of a DNA sequence. Epigenetic mechanisms involve enzymes that control genome functioning in a variety of complementary ways. These include DNA methylation, histone post-translational modifications and microRNA expression. This orchestrated control allows our cells to synchronise with each other and organisms to adapt to changing environmental situations.

These epigenetic mechanisms have also been described as drivers in neurodegenerative and psychiatric disorders. Epigenetic mechanisms control the formation of the nervous system and the creation and differentiation of neurons during development and continue way after birth and into adult life. DNA and histone modifications have been associated with memory, cognition and neuronal death both in animal models and human patients[1].

Histones are a family of proteins that associate with DNA in the nucleus and help compress it into chromatin. Chromatin is the combination of DNA and protein that make up the cell nucleus. Histones can be chemically modified through the activity of enzymes. These post-translational changes can impact gene expression by, for example, altering chromatin structure. The best understood of these changes is acetylation and de-acetylation which are associated with memory formation. Another type of histone modification is methylation and demethylation which has been shown to affect cognition and memory. These modifications are reportedly involved in Alzheimer's disease as well as psychiatric diseases such as schizophrenia[2] and autism[3] and neurodevelopmental disorders like Kabuki[4] or Kleefstra[5] syndromes.

The post-translational changes executed by these epigenetic mechanisms are only part of a more elaborate waltz however. Epigenetic enzymes are often the building blocks for populated transcription factor (TF) complexes which regulate the expression of genes in the brain. Two enzymes in the brain that modulate this gene expression and feature in many of these TF complexes are histone deacetylase 2 (HDAC2) and LSD1. Today, these enzymes are leading targets for drug discovery. The focus of research at our company is on LSD1.

As disciplines, psychiatry and neurology were born at different times, but both identify symptoms and syndromes in categorising diverse mental dysfunctions. Medical intervention in CNS diseases today still relies on a symptom-based diagnosis of disease. This descriptive phenomenological approach was important for these disciplines to progress, but today it is challenged by our new knowledge about the heterogeneity of these diseases.

Epigenetic targets are starting to appear on the scene to inform drug development and deal with this heterogeneity. In particular, LSD1 is emerging as a molecular hub where several defective pathways appear to be converging to produce a disease phenotype. For the first time, scientific findings around LSD1 open the door to possible personalised pharmaceutical interventions in certain genetically defined CNS patient populations.

Schizophrenia is one example. In this disease, the chromatin modifying enzymes SETD1A and LSD1 work together to balance the methylation of histones. SETD1A is encoded by the *Setd1a* gene. SETD1A is part of a larger protein complex called Set1/COMPASS. A large international gene sequencing study of more than 4,000 people diagnosed with schizophrenia and over 9,000 controls found that rare natural loss-of-function mutations in the *Setd1a* gene were associated with big increases in the risk of schizophrenia[6]. Similar mutations of other genes associated with the COMPASS protein complex have been associated with the risk of autism[7].

SETD1A is associated with gene activation. By comparison, LSD1 counteracts this activity and down-regulates gene expression. While SETD1A activity appears to be essential in humans for normal brain and neural development, overactive LSD1 after birth leads to the down-regulation of beneficial neuronal plasticity genes, blocking or slowing brain development and neural function.

A recent study

In a recent study, a scientific team led by Joseph Gogos at Columbia University in New York City, US, characterised a mouse model of schizophrenia in which one of two copies of the *Setd1a* gene could be switched on or off[8]. When one copy of the gene was switched off, physical patterns of connections between neurons and brain regions were altered. In addition, mouse memory was affected and the animals found it more difficult to learn how to find their way out of a maze. When the gene was switched back on in adulthood, memory and learning function was partially restored.

The authors further report that pharmaceutically inhibiting LSD1 in adult mice with a deficient *Setd1a* gene was successful. In the study, they used Oryzon Genomics' LSD1 inhibitor iadademstat. It showed that LSD1 inhibition compensated for the deficit in SETD1A enzymic activity, resulting in a full rescue of prefrontal cortex axonal branching and axonal navigation deficits. It also corrected behavioural and cognitive abnormalities. This study suggests that LSD1 inhibition in genetically defined subpopulations of patients with schizophrenia with a *Setd1a* gene mutation may be effective.

LSD1 inhibition has also been shown to enhance cognitive function in non-*Setd1a* gene based settings. For example, Takeda Pharmaceutical Company Ltd reported that LSD1 inhibition with one of its molecules partially restored learning function in mice with N-methyl-d-aspartate (NMDA) receptor hypofunction, one of the most prevalent models of schizophrenia[9]. Another Takeda LSD1 inhibitor normalised hippocampal memory defects in a mouse model for Kabuki syndrome after two weeks of treatment[10]. Kabuki syndrome is a rare neurodevelopmental and multisystem disorder caused by mutations in the *KMT2D* gene.

At Oryzon, we have also reported promising preclinical data for our small molecule LSD1 inhibitor vafidemstat in the CNS setting. This study was conducted in the senescence Accelerated Mouse Prone 8 (SAMP8) model for accelerated ageing and Alzheimer's disease. Vafidemstat was shown to correct memory deficits, reduce aggression, improve social behaviour and modulate gene expression in the prefrontal cortex [11]. Also, the compound improved cognition in models of Huntington's disease. Vafidemstat is in Phase 2 development for several CNS indications, and has been shown to reduce aggression and agitation in Phase 2a trials in autism spectrum disorder, adult attention deficit hyperactivity disorder, borderline personality disorder and Alzheimer's disease patients.

It is important to note that the connection between LSD1 and schizophrenia is not just an oddity restricted to LSD1's specific interplay with SETD1A mutant proteins. Emergent evidence points to a broader involvement. A recent genome-wide association study revealed that a single nucleotide polymorphism located near the gene *miR-137* which encodes for an RNA molecule, has a strong association with schizophrenia. A transcription factor known as REST and LSD1 regulate *miR-137* expression. At the same time, *miR-137* regulates LSD1 protein expression. LSD1 inhibitors could therefore potentially be used to mitigate excessive LSD1 activity in schizophrenia and upregulate *miR-137* expression.

Throughout this article, we have specifically focused on personalised, or targeted, approaches to treating CNS diseases. It is clear that this approach is receiving more and more attention. Another example comes from the work of Zhen Yan and colleagues at the University of Buffalo in the US. Dr Yan's group has characterised a mouse model with *Shank3* gene deficiencies which closely parallels a rare human form of autism known as Phelan-McDermid syndrome. The *Shank3* gene encodes a scaffolding protein at glutamatergic synapses. The University of Buffalo research team showed that administration of the drug romidepsin, an HDAC inhibitor, induced a robust and prolonged rescue of autism-like social deficits in the mice when a variety of drugs for psychiatric disorders failed to do so[12].

As mentioned, HDAC2 and LSD1 are often part of the same brain complexes and, interestingly, the same group reported in 2019 that a brief treatment of *Shank3*-deficient mice with a highly potent LSD1 inhibitor described in one of Oryzon's granted patents, also led to the rescue of core symptoms of autism including electrophysiological abnormalities, social deficits and repetitive behaviours.

Towards personalised medicine in CNS

Patients who are affected and will ultimately be treated with personalised therapies often constitute sub-populations of larger patient groups. For example, cases of schizophrenia caused by mutations in the *Setd1a* gene are estimated to represent only 0.2 to 0.8% of all schizophrenia cases and 0.1% of children with severe developmental disorders[13]. A key challenge in developing drugs for these disorders is finding the relevant patients. Patients with inherited mutations of *Setd1a* have been identified in the Amish founder population in Pennsylvania, US. Our company has entered into a collaboration with a research group at Columbia University in New York City to conduct a pilot study to characterise the psychometric profile of these individuals and to determine their different degrees of cognitive impairment. This study will inform the design of future clinical studies of vafidemstat in this patient population.

As discussed earlier, Phelan-McDermid syndrome, which is caused by the underexpression of the *Shank3* gene, can lead to severe intellectual disability, delayed speech, repetitive behaviour and autism[14]. Although people with this disorder represent only 1 to 2% of all cases of autism spectrum disorder, there is a clear medical need for a new treatment. Currently, there are only limited symptomatic treatments for the disease such as anti-psychotic drugs.

The Institute of Medical and Molecule Genetics of the La Paz University Hospital in Madrid, Spain has identified the *Shank3* mutation in some 200 Spanish and Latin American patients with Phelan-McDermid syndrome in recent years. In June 2020, Oryzon entered into a collaboration with the institute to stratify patients with this syndrome to inform a clinical study with vafidemstat in this disease. We are combining this targeted approach to personalised medicine in rare CNS conditions with broader Phase 2b trials of vafidemstat in schizophrenia and borderline personality disorder in the general population.

Conclusion

LSD1 is a fascinating epigenetic target. It is also an example of how we may start untangling the complex bundling of behavioural and cognitive symptoms of rare conditions like Phelan-McDermid syndrome or *Setd1a*-related schizophrenias. An added benefit is that this molecular dissection will no doubt provide a better insight into the bigger CNS indications such as schizophrenia, autism and Alzheimer's disease. Propelled by this approach, therapies for CNS diseases could, for the first time in decades, move beyond the treatment of symptoms and towards disease modifying cures.

Terms that are relevant to this article:

Chromatin: A complex of DNA and proteins of which chromosomes consist.

DNA Methylation: The addition of a methyl group to the DNA. It usually occurs at DNA cytosine nucleotides followed by guanosine (C-phosphate-G, CpG) and represses gene expression.

Epigenetics: heritable changes in gene expression that do not result from changes in actual gene sequences.

Gene Expression: The production of messenger RNA (mRNA) using a DNA gene sequence as a template. The mRNA will (after various sorts of processing) be translated into a protein.

Genome: All the DNA in an organism or cell, especially with reference to the total sequence of nucleotide bases, or 'letters' of the genetic code.

Histones: Proteins upon which DNA is tightly wound and whose function is to condense and package DNA in the nucleus.

Histone Modifications: Post-translational addition or subtraction of any one of several chemical groups to an individual amino acid of a histone. Depending on the chemical group involved, the modification is called methylation (addition of a methyl group), acetylation (addition of an acetyl group), phosphorylation, ubiquitination and sumoylation. These modifications can dramatically affect the electrical and other properties of the chromatin, and play a major role in gene regulation.

Histone deacetylases (HDACs): Enzymes involved in deacetylation of histones. A large and complex super-family of proteins which are highly conserved in their sequence.

Histone deacetylase 2 (HDAC2): An HDAC enzyme belonging the HDAC Class-1 family. Implicated in CNS functions and memory.

Histone demethylases (KDMs): Enzymes involved in demethylation of histones. A large and complex super-family of proteins divided in two main groups. LSD1 and LSD2 are the only known members so far of the family known as FAD-dependent KDMs.

Lysine Specific Demethylase 1 (LSD1): The first histone demethylase discovered. It has a key role in differentiation of the CNS and plays a role in memory and cognition.

MicroRNA (miRNA): Single-stranded, non-protein coding RNAs about 21-24 nucleotides in length expressed in plants and animals. They regulate gene transcription by binding to the target gene's 3' untranslated region causing the block of transcription or promoting degradation.

Transcription: Biochemical process in which an intermediary molecule called messenger RNA is generated based on the genetic information of the DNA.

Transcription Factor: A protein that is involved in the mechanisms by which genes are transcribed. They work often in group with other proteins in the so-called Transcriptional Complexes.

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