Commentary: Oliver Rausch

RNA epigenetics is coming of age

RNA epigenetics describes the role of chemical modifications of RNA in controlling its post-transcriptional fate. All known RNA species are known to be modified by specific enzymes, or enzyme complexes ('readers'), and to date, over 150 different modifications have been described, ranging from simple methylation to highly complex modifications involving multiple enzymes. The vast majority of modifications have been identified in non-coding RNAs such as ribosomal RNA and transport RNA, reflecting one of their key roles in regulating RNA structure and stability.

In this article, we discuss the role of RNA epigenetics in medicine with specific reference to the METTL3 enzyme as a target for cancer. We also look beyond cancer to potential applications for the technology in the fields of infectious and rare diseases. These issues were discussed at an RNA epigenetics conference in Cambridge, UK on 17 to 19 September, organised by Storm Therapeutics Ltd.

There are many events that led up to the birth of RNA epigenetics. Arguably, a turning point in the field was the discovery that methylation of mRNA on the 6-position of adenosine (m6A) was a pivotal mechanism for the control of translation and degradation of mRNA. The subsequent identification of specific m6A-binding proteins ('readers') that are key in mediating the effects downstream of m6A, as well as of specific demethylases that delete the m6A mark ('erasers'), highlighted the dynamic nature of the m6A modification and inevitably invited parallels with epigenetic regulation of chromatin[1].

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It became clear that RNA is subjected to an intricate level of post-transcriptional control by RNA modifications, allowing cells to rapidly adapt their transcriptome to external stimuli in order to meet new requirements and functions. It is this new regulatory function of RNA modifications, and the excitement stirred by epigenetic drugs such as EZH2, HDAC and bromodomain inhibitors that has put RNA epigenetics firmly into the spotlight for drug discovery.

Over the past decade the interest in targeting RNAs for the development of new drugs has risen sharply. RNAi and antisense approaches led the field, but have been hampered by technical challenges and the difficulty of delivering them to the appropriate tissues. RNA epigenetics offers a new opportunity for targeting RNA therapeutically, as it provides an avenue for the development of traditional small molecule drugs acting via RNA. Enzymes constitute a proven target class for successful small molecule drugs, so targeting the enzymes responsible for key modifications allows direct modulation of RNA function. This idea is gaining further traction as it becomes clear that many modifications and their corresponding enzymes are dysregulated in disease[2,3].

Although most of the current evidence points to cancer as the therapeutic area of choice for modulators of RNA modification, undoubtedly any disease that is driven by inappropriate activation, expansion or differentiation of particular cell types presents a potential therapeutic opportunity for such drugs. Already, strong evidence is emerging that RNA modifications are equally important in neuroscience, metabolism and immunology, raising expectations that this new mode of action may also lead to new opportunities for the treatment of other diseases.

Highlights from the conference

RNA epigenetics is a brand new field of biology, and as such, too early to be targeted by traditional large pharma. However, it presents a perfect hunting ground for innovative, ground-breaking start-up companies. Over the past three years, several new and well-funded biotechs have emerged with a focus on targeting RNA modifications, namely Storm Therapeutics in Cambridge, UK and the US companies Accent Therapeutics Inc and Gotham Therapeutics Corp. At the Cambridge conference in September, Storm Therapeutics' founders Tony Kouzarides and Eric Miska, gave some insights into how each of these companies is approaching the challenges, and what progress they have made. All three companies have declared that they are targeting the m6A writer enzyme METTL3, responsible for the most prevalent and best studies of RNA modification, m6A.

Storm Therapeutics is targeting a number of additional undisclosed methyltransferases, whereas Accent Therapeutics has made significant progress in targeting another enzyme, ADAR1, an RNA A-to-I editor. Gotham Therapeutics appears to have a strong focus on the m6A modification, targeting not only the writer enzyme METTL3, but also eraser demethylases and YTH reader proteins.

What will ultimately validate the field and lift it onto the radar of pharma companies is a compelling demonstration of therapeutic efficacy and safety of molecules targeting RNA modifications. METTL3 may turn out to be the pathfinder target providing this evidence. In a presentation at the Cambridge conference, Storm Therapeutics, for the first time, disclosed data from its METTL3 programme, describing small molecule inhibitors of METTL3 that are orally bioavailable and show pronounced anti-tumour efficacy in therapeutically relevant proof of concept animal models of acute myeloid leukaemia (AML). Importantly, Storm's molecules demonstrate a consistent pharmacological

audit trail across biochemical systems, cell-based pathway and phenotypic assays, *in vivo* biomarkers and functional efficacy.

Storm's data also demonstrated that small molecule inhibition of METTL3 produces the same effects and phenotype previously described using genetic models, validating METTL3 as a small molecule target for cancer. While it is too early to draw final conclusions on the safety of targeting METTL3, Storm was able to dose its molecule continuously for three weeks without any signs of adverse events. Storm is now building on the story in AML by evaluating its molecules in a range of solid cancers driven by m6A methylation and aims to enter clinical trials with a candidate molecule in 2021.

The first lesson discussed at the conference is that targeting METTL3 is not easy. While all three companies employed a high throughput screen (HTS) to identify chemical starting points, Storm appears to be the only company which succeeded in this approach. Accent described a knowledge-based approach employing *de novo* design, while Gotham used a fragment screen after its HTS failed to identify progressable chemistry.

A common issue not only for METTL3, but also other RNA methyltransferases, appears to be a high false positive rate in all high throughput screening campaigns. Unsurprisingly, all three companies highlighted the importance of orthogonal biophysical assays during hit characterisation, and are employing METTL3 crystallography to drive lead optimisation. Both Storm's and Accent's inhibitors bind to the SAM pocket of METTL3 and are SAM competitive, resulting in a significant drop-off in activity from biochemical to cellular systems, thus requiring significant optimisation of potency and residence time to achieve efficacy. Nevertheless, Storm was able to go from HTS to *in vivo* proof of concept in less than 18 months.

A major challenge common to all potential targets in the field is the lack of quantitative technologies to measure modifications as biomarkers. A key emerging technology that addresses this challenge, and that is employed by all three companies, is mass spectrometry. Storm has made significant investments in this area and established a mass spectrometry platform fully dedicated to RNA modification analysis, including a ground-breaking approach that allows quantitative analysis of RNA modifications in a sequence-specific context, enabling the use of RNA modifications as pharmacodynamic biomarkers.

Beyond RNA mass spectrometry, exciting novel sequencing methodologies based on chemical or enzymatic recognition of specific RNA modifications or even allowing unbiased detection of modifications through nanopore sequencing, are now emerging that will ultimately allow monitoring of RNA modifications in patients, and their use as stratification biomarkers.

Several talks by academic leaders at the conference highlighted exciting opportunities for METTL3 inhibitors beyond cancer. For instance, Stacey Horner from Duke University Medical Center, US discussed the important role of m6A in regulating the life cycle of and controlling the immune response to RNA viruses such as hepatitis C, dengue and Zika. Jeannie Lea from Harvard Medical School, US discussed silencing of the second X chromosome in females by the long non-coding RNA Xist, and her efforts to reactivate transcription of the gene MECP2 from the inactive X chromosome (Xi) as a potential treatment strategy for RETT syndrome. RETT syndrome is a rare disease in girls, caused by a heterozygous mutation in MECP2 on the active X chromosome. She showed how re-activating expression of the healthy MECP2 gene can revert symptoms in a mouse model of RETT syndrome. Xist RNA is m6a modified and m6A is implicated in the maintenance of Xist mediated X chromosome inactivation. It is thus intriguing to speculate that inhibitors of METTL3 could be used to reactivate MECP2 transcription from Xi and treat RETT syndrome.

Future perspectives

Whilst all the eyes are currently on METTL3 inhibitors, it is only a matter of time until pharmacological inhibitors of other RNA modifying proteins will become available. Two of the hot drug targets on the scene are the group of YTHDF proteins acting as specific m6A 'readers' and the RNA A-to-I editor ADAR1.

Genetic knockouts of one or more of the YTHDF proteins mimic the phenotype caused by inhibition of METTL3, and selectively inhibiting YTHDF proteins may present a potentially more specific approach to targeting m6A biology. Computational analyses show that YTH domains are druggable with small molecules in principle, and several groups, including Gotham Therapeutics, have reported the identification of early stage inhibitors of YTH-m6A binding.

ADAR1 has been shown to be a specifically essential enzyme in a particular set of tumours and cancer cells characterised by active type I interferon signalling. Their characteristic interferon-stimulated gene signature marks these tumours as vulnerable to ADAR1 inhibition and could be used as a stratification marker for patient selection. In addition, inhibiting ADAR1 in other cancer cells appears to trigger their interferon response, and render these cells susceptible to attack by the immune system. This raises the exciting prospect of combining ADAR1 inhibitors with checkpoint inhibitor drugs such as PD1 or PD-L1 antibodies to boost the response to these drugs.

Targets like METTL3 and ADAR1 may only be the beginning. Based on the rapid growth of our understanding of RNA modifications, the message from the field is clear: watch this space - a plethora of new drugs may be just round the corner.

References:

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