The viral invisibility cloak

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Previously considered as a silent virus, the bovine leukaemia virus (BLV) produces small molecules - microRNAs - invisible to its victim’s immune system. The virus may thus produce these small molecules while remaining hidden in the host cell. This explains why so far BLV was considered to be silent in tumours. These viral microRNAs may thus be the key to understanding how BLV induces cancerogenesis without triggering an immune response in the organism it is infecting. A study published in PNAS by the Animal Genomics Research Unit of GIGA at the University of Liège, headed by Michel Georges looks into this in more depth.

As its name indicates, the bovine leukaemia virus (BLV) naturally infects cattle and triggers the development of leukaemia, a cancer of the blood. Although eradicated in Belgium, this disease still affects many countries, particularly in North America and Eastern Europe. 'In the United States and Canada for example, 80% of dairy herds are infected,' reports Anne Van den Broeke, Scientific Research Worker at the FNRS and project leader within the Animal Genomics research unit at the ULg, led by Michel Georges. 'Although it doesn’t represent a danger to humans, this disease is a major concern in terms of animal health and causes considerable economic losses to these countries,' she continues. Avenues for research may lead to the development of a vaccine or an early diagnostic test, which are major issues for these countries. Moreover, BLV shares many similarities with a human virus, which is endemic in Japan, the Caribbean and Latin America, and causes leukaemia in humans. 'Clearly, therefore, it is interesting to study BLV as a tool to better understand how the equivalent human virus operates' continues Van den Broeke.

Finally, studying this virus helps unraveling the fundamental mechanisms which enable viruses to induce anarchic proliferation of cells, i.e. cancer. 'BLV has been studied for a long time and we know that it is capable of coding for oncogenes, cancer-promoting proteins. At the level of the tumour cell however, these proteins are absent and the previously-documented viral genes are found to be silent.'

Sheep: a uniquemodel

'Once infected by BLV, it takes about ten years on average for cattle to develop leukaemia,' continues Van den Broeke. A characteristic which doesn't make it any easier to study the pathogen's mode of action! Fortunately, scientists don't lack the imagination and ingenuity required to overcome this type of obstacle. As an alternative for studying the virus in its natural host, they use sheep as a research model. When sheep are infected with BLV, the disease progresses much more rapidly than in cattle, and leukaemia develops after an average of eighteen months in all infected sheep (while in infected cattle, only a small percentage, about 2 to 5%, develop leukaemia).

In the context of a recent study published in the PNAS (1) journal, Anne Van den Broeke and her team combined observations in this interesting animal model with a cutting edge technology: deep sequencing. 'This technology revealed that BLV produces high levels of a particular class of small non-coding molecules, called microRNAs. These molecules may be one of the keys to understanding how the virus causes cancerogenesis' she reveals.

When technology pushes the limits

What is deep sequencing? The genetic material of living creatures can be compared to a code made up of four letters, the order of which determines which messages are communicated. 'High-throughput sequencing
consists of detecting and decoding these messages without a prior knowledge of the composition of the genome or transcriptome. It may be compared to the generation of millions of words or short sentences which bioinformaticians analyse in order to distinguish the words which mean something from those which don't', explains Van den Broeke.

'Until recently, 2% of mammalian genomes were believed to be important (called "genes" or "coding genome") and the remaining 98% was considered useless. But we are starting to understand that the non-coding part of the genome is critical as well, as it plays a significant role in gene regulation'. Although earlier methods failed to spot them, microRNAs such as those discovered by the ULg researchers in BLV could be identified using deep sequencing techniques. These small non-coding viral RNAs are non-immunogenic, i.e. they do not induce a response from the infected animal's immune system. 'The virus may thus produce these small molecules while remaining hidden in the host cells. This explains why so far BLV was considered to be silent in tumours.' explains Van den Broeke. Although scientists had detected the presence of BLV in these tumours, there was no evidence of the virus' activity.
Preventing expression of these microRNAs

'\textit{We have identified a cluster of ten viral microRNAs and we think they may have an impact on the virus host cells, leading them towards malignancy. These molecules may also play a critical role in inhibiting the expression of other viral components and as a result may contribute to virus silencing and escape from the host’s immune system}', indicates Van den Broeke. In light of these hypotheses, the researchers have come up with the suggestion that preventing viral microRNA production may prevent the development of bovine leukaemia. 'If this is the case, a genetically manipulated virus could serve as the basis for a vaccine against this disease and prepare the bovine immune system to react against the infection of a natural virus', she continues.

The next steps in this research will be to study the behaviour of a BLV virus which can no longer produce the small RNAs, and to monitor the disease progression in sheep which are infected by this virus compared to those who carry the natural strain. 'We will also try to better understand the function of these small molecules in tumor onset and progression', Van den Broeke adds.

Where cancerology and animal genomics meet

This work is a good example of interdisciplinary collaboration combining research areas such as cancerology, viral pathogenesis and animal genomics. In terms of the human virus which is equivalent to BLV, the Liège researchers have tried to identify whether it also produces similar microRNAs. 'Preliminary results do not suggest a same mode of action of the human virus at the leukemic stage. However, leukaemia takes several decades to appear after the initial infection in humans', explains Van den Broeke. 'Non-coding RNA molecules may be produced by the virus during early stages of infection rather than 20 or 30 years later, when the leukaemia becomes apparent. Obtaining human samples from very early stages is challenging, but that’s what we will try to verify', concludes Van den Broeke.

The recent discovery of these viral microRNAs in BLV represents a considerable step forward in understanding the mechanisms which underlie its action within host cells and raise a number of new questions. Now that researchers have succeeded in identifying one of the keys to the secret of BLV, they plan on exploring the different hypotheses it raises, with the ultimate goal of one day shedding light upon the mystery surrounding its mode of action.

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