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Expression of Artificial microRNA by Oncolytic Measles Virus – a New Strategy to Overcome Resistance Mechanisms and Enhance Tumour Cell Killing

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Oncolytic viruses have emerged as promising therapeutics for the treatment of cancer. The rising interest in oncolytic virotherapy is reflected by the increasing number of preclinical and clinical trials testing genetically modified oncolytic viruses from distinct virus platforms. Due to their outstanding safety profile and oncolytic potency, measles vaccine strains constitute promising oncolytic virotherapeutics and are currently tested in eight clinical trials. Although reported results are quite encouraging, trials have also shown the constraints of oncolytic virotherapy. Major obstacles include hampered delivery of oncolytic viruses to tumour tissue, limited tumour cell specificity as well as incomplete tumour cell killing which can be due to resistance mechanisms of cancer cells. Measles virus-mediated delivery of microRNAs was envisioned as a new strategy to overcome respective resistance mechanisms. microRNAs are small RNA molecules which downregulate thousands of cellular genes and could be exploited to suppress resistance factors in order to increase viral growth and to enhance the efficacy of measles virus. This study aimed at providing the proof-of-concept that oncolytic measles viruses are capable of delivering functional microRNAs.

Oncolytic measles viruses were engineered to express artificial microRNAs in form of a primary microRNA mimic (MeVami-X) designed to enable efficient microRNA processing. Cells infected with the respective viruses expressed primary microRNA transcripts as well as mature microRNA, verified by RT-PCR and qPCR, respectively. However, the functionality of virus-derived microRNA could not be shown in a luciferase-based assay, presumably due to low microRNA expression levels. Therefore, an alternative microRNA expression cassette encoding primary microRNA-122, which had been shown to produce considerable amounts of microRNA-122 in the context of other cytoplasmic RNA viruses, was cloned into measles virus genomes to test whether respective viruses can generate higher microRNA expression levels. The expression of primary and mature microRNA-122 was verified by RT-PCR and qPCR, respectively. In fact, the expression levels of microRNA-122 exceeded the expression of artificial microRNA derived from the MeVami-X cassette. The highest microRNA expression level was determined for microRNA-122 deriving from measles virus harbouring the microRNA expression cassette upstream of the N gene, which is consistent with the measles virus transcription gradient. The comparatively high expression of respective microRNA-122 translated into specific target suppression in the luciferase-based assay, suggesting that the amount of expressed microRNA is critical for target suppression. Overall, expression levels of viral microRNA are in the range of endogenous microRNA expression levels which suggests that measles virus-derived microRNAs sufficiently mediate target suppression.

However, when compared to other oncolytic viruses, the expression levels of measles virus-derived microRNA are low. In the context of other RNA viruses with cytoplasmic replication cycles, the RNase III Drosha, which commonly facilitates the first step of canonical microRNA processing in the nucleus, has been shown to translocate to the cytoplasm upon viral infection enabling the cytoplasmic processing of virus-derived microRNA. However, it could be demonstrated that Drosha remains in the nucleus during measles virus infection suggesting that Drosha does not catalyse the cleavage of measles virus-derived primary microRNA in the cytoplasm. The translocation of Drosha to the cytoplasm is also induced by double-stranded RNA, a common viral pathogen-associated molecular pattern, which implicates a role for Drosha in the antiviral response. Cytoplasmic RNA viruses which have been shown to induce the translocation of Drosha are known to generate considerable amounts of double-stranded RNA during their replication cycle, whereas measles virus only generates low amounts of double-stranded RNA. It may be concluded that measles virus does not induce the translocation of Drosha as it does not produce relevant amounts of double-stranded RNA. This might be considered as a mechanism to circumvent the antiviral response. As Drosha is located in the nucleus during measles virus infection, whereas the primary microRNA is transcribed in the cytoplasm, it is unknown how virus-derived microRNA is processed. Restricted microRNA processing may explain the comparatively low expression levels of measles virus-derived microRNA.

The expression of functional microRNA by measles virus provides the proof-of-concept that oncolytic measles viruses are capable of delivering functional microRNA. In the future, the expression of microRNAs can be exploited to suppress resistance factors, to increase viral growth and to enhance tumour cell killing. Recombinant viruses expressing selected miRNAs should be assessed in vitro and in vivo in terms of their anti-tumour efficacy and their safety level. With regard to the biogenesis of measles virus-derived microRNAs, further studies are required to elucidate the underlying mechanisms. New insights could possibly be translated into enhanced processing of microRNAs and therefore contribute to an increased anti-tumour efficacy of microRNA-armed measles virus.

In summary, the delivery of microRNAs constitutes a new arming strategy for measles virus which may help to overcome one of the remaining hurdles in oncolytic measles virotherapy. The data presented in this work may have implications for future clinical applications and thus warrant further investigation.