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НИЗКАЯ ЭКСПРЕССИЯ ВИРУСНЫХ микроРНК В МАКРОФАГАХ И НЕЗРЕЛЫХ В-КЛЕТКАХ ПРИ ЛАТЕНТНОЙ ИНФЕКЦИИ ГИГРОМИЦИНУСТОЙЧИВОГО ГАММАГЕРПЕСВИРУСА-68 МЫШИ[#]

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Гаммагерпесвирус-68 мыши (murine gammaherpesvirus 68, MHV68) латентно инфицирует главным образом В-клетки и вызывает у лабораторных мышей лимфому, напоминающую по симптоматике гаммагерпесвирусные заболевания человека. Для изучения молекулярного механизма вирусной инфекции и того, как вирусные детерминанты контролируют клетку и в итоге вызывают онкогенез, необходимы легкодоступные латентно инфицированные клеточные линии. Для *in vitro* исследований латентности MHV68, как и других гаммагерпесвирусов, доступны только две системы культивирования: созревающие В-клетки и макрофаги. В связи с этим актуальна задача расширения репертуара клеточных линий, латентно инфицированных MHV68. В проведенном исследовании получено несколько клонов линий незрелых В-клеток и макрофагоподобных клеток с латентным фенотипом. Устойчивый к гигромицину рекомбинантный вирус MHV68 был выделен из лабораторной линии латентно инфицированных клеток HE2.1 и размножен под селекцией гигромицина для получения стабильных клеточных линий, несущих вирусный геном. В субклонах этих клеточных линий анализировали экспрессию вирусных микроРНК с помощью количественной ПЦР TaqMan и оценивали экспрессию литического вирусного транскрипта M3. Клеточные линии сохраняли вирусный геном в виде эписомы, что показано с помощью ПЦР-анализа с расщеплением-циркуляризацией. Несмотря на то что полученные латентно инфицированные клеточные линии экспрессировали вирусные микроРНК на уровнях, не превышающих таковые в родительской клеточной линии, их можно рассматривать как альтернативный инструмент для изучения механизмов латенции и идентификации мишеней микроРНК.

Ключевые слова: MHV68, мышиный гаммагерпесвирус 68, латентная стадия, клеточная линия, вирусные микроРНК, ПЦР с расщеплением-циркуляризацией

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Latent Macrophage and Immature B Cell Lines Generated with Hygromycin-Resistant Murine Gammaherpesvirus 68 Genome Expresses Modest Levels of Viral miRNAs

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Murine gammaherpesvirus 68 (MHV68) establishes latency mainly in B cells and causes lymphomas reminiscent of human gammaherpesvirus diseases in laboratory mice. To study the molecular mechanism of virus infection and how the viral determinants control cell and eventually cause tumorigenesis, readily available latently infected cell lines are essential. For *in vitro* MHV68 latency studies, only two cell culture systems have been available. Gammaherpesviruses are known to infect developing B cells and macrophages, therefore we aimed to expand the MHV68 latently infected cell line repertoire. Here, several latently infected immature B cell and macrophage-like cell line clones were generated. Hygromycin-resistant recombinant MHV68 was isolated from a laboratory-made latent cell line, HE2.1, and propagated to develop stable cell lines that carry the viral genome under hygromycin selection. Subclones of these cells lines were analyzed for viral miRNA expression by TaqMan qPCR and assessed for expression of a lytic viral transcript M3. The cell lines maintain the viral genome as an episome shown by the digestion-circularization PCR assay. Latently infected cell lines generated here do not express viral miRNAs higher than the parental cell line. However, these cell lines may provide an alternative tool to study latency mechanisms and miRNA target identification studies.

Keywords: MHV68, gammaherpesvirus latency, latent cell line, murine gammaherpesvirus miRNA, digestion-circularization PCR