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Effect of LSDV008 gene knockout on the cultural properties of recombinant lumpy skin disease virus

Lumpy skin disease virus (LSDV) is a poxvirus that causes a severe systemic disease in cattle and is rapidly expanding its geographic boundaries. Lumpy dermatitis (LD) is characterized by fever, nodules on the skin, mucous membranes, and internal organs. The disease can cause emaciation with swollen lymph nodes and sometimes death. In recent years, the disease has become endemic in various parts of Asia, causing significant economic damage to livestock production. Since there are no specific treatments for LSD, vaccination is the most effective way to control and eradicate the disease. The most complete immunity can be obtained by vaccination with live attenuated vaccines. Attenuation through long passages is associated with random mutations in the genome, and the mechanism of attenuation remains unclear. Targeted removal of virulence genes in the viral genome by genetic engineering is the most promising direction in the creation of attenuated poxviruses. The LSDV008 gene encodes a protein similar to the γ-interferon receptor and is apotential virulence gene for the LSD virus. In these studies, we studied the effect of deletion of the LSDV008 gene on the cultural properties of the recombinant Atyrau-B virus. The parental LSD virus Dermatitis nodulares/2016/Atyrau/KZ (Atyrau-KZ) was used as a control. As a result of the studies, it was found that the gene knockout did not affect the replication activity of the recombinant Atyrau-B virus *in vitro*. The recombinant virus accumulated in cell cultures in the same titers as the parent virus. The most sensitive cell systems for the reproduction of Atyrau-KZ and Atyrau-B LSD viruses are lamb testicle (LT), bull kidney (MDBK) and saiga kidney (SK) cell cultures, which can be used to obtain viral mass in further scientific research.

Keywords: lumpy skin disease virus, cell culture, cultivation, virus titer, replication, infectious activity, gene knockout, attenuation.

Introduction

Lumpy skin disease is an acute, subacute, chronic, less often latent disease of cattle characterized by fever, development of nodular skin lesions, skin necrosis, generalized lymphadenitis, and edema of the ventral parts of the body and extremities. The disease can be seen in buffaloes, cattle, giraffes and impalas.

The causative agent of the disease is the bovine lumpy skin disease virus (LSDV), which has an antigenic relationship with strains of viruses that cause pox in sheep and goats, which differ at the genetic level, and together with it form an independent genus *Capripoxvirus*, family *Poxviridae*.

LSDV is one of the largest known human and animal viruses, virions have a spherical shape, the diameter of the virion is 300-450 nm. LSDV genomic DNA has a size about 150 thousand bp.

In order to prevent lumpy skin disease, active attenuated vaccines are considered effective [1]. They have been used in veterinary practice for many years and have also shown their safety, reliability and the ability to guarantee long-term protection. The development of attenuated lumpy skin disease vaccines follows a traditional approach. In the process of prolonged passaging of the virus in the cells of permissive and non-permissive hosts, mutations appear in the genome, which lead to a decrease in virulence. The mechanism of attenuation remains unclear. In this regard, there is a threat of reversion to virulence [2-4].

An alternative method for attenuating viruses has become site-directed mutagenesis. In recent years, the creation of attenuated poxviruses has increasingly begun to use genetic engineering methods, namely, the method of inactivation of virulence genes [5-8]. Potential virulence genes that confer increased pathogenicityand increase the replication activityof capripoxviruses in immunocompetent hosts have been identified by genome sequencing and annotation [9]. Only a few genes of capripox viruses have received experimental confirmation of their functions. Pilot studies were conducted in which two putative

immunomodulatory genes (ORF005 and ORF008) were deleted separately from the genome of a virulent LSDV field isolate. As a result of the study, it was determined that the deletion of the genes resulted in the manifestation of avirulent LSDV phenotype in cattle, but was safe in sheep and goats. Also, other authors obtained a strain of sheeppox virus (SPPV) in which the ORF019 gene was deleted. As a result of infection of lambs with the virus, data were obtained that indicate that the ORF019 gene is an important determinant of SPPV virulence in sheep [10-12].

Previously, we obtained a recombinant LSD virus Atyrau-B with a deletion of the LSDV008 gene. This gene encodes an interferon gamma receptor-like protein (IFN-γ) and is apotential virulence gene. Using the myxoma virus as an example, it was shown that this gene is expressed early after infection and remains in the supernatants of infected cells until late postinfection periods, inhibits the binding of IFN-γ to its cellular receptor, thereby eliminating its antiviral activity [13]. Deletion of genes in the viral genome should not affect their replication activity in vitro. In these studies, we studied the effect of deletion of the LSDV008 gene on the cultural properties of the recombinant Atyrau-B virus.

Experimental

LSDV viruses were used in the study: virulent strain Dermatitis nodulares/2016/Atyrau/KZ (Atyrau-KZ), recombinant Atyrau-B with LSDV008 gene knockout; and the following cell cultures: primary trypsinized lambs testicle cells (LT), transplantable bovine kidney cell line (MDBK), transplantable African green monkey kidney epithelial cell line (Vero), transplantable saiga kidney cell line (SK), transplantable calf testicular cell line (CT).

The sensitivity of cell cultures to viruses was determined by the method of successive passages. A monolayer cell culture was infected with Atyrau-KZ and Atyrau-B viruses and cultivated until a 100% cytopathic lesion of the monolayer appeared. Infected cells were lysed by double freeze-thaw. The infectious activity of viruses was determined by microtitering in LT cell culture. The calculation of infectious activity was carried out according to the method of Reed and Mench and expressed in lg TCD50/cm^3. Accounting for the results of microtitering was carried out on the 10th day of incubation.

Results and Discussion

To study the cultural properties of the Atyrau-B virus, five successive passages were carried out in various cell cultures. The parent Atyrau-KZ virus was used for comparison. The research results are presented in Figure 1 and Table.

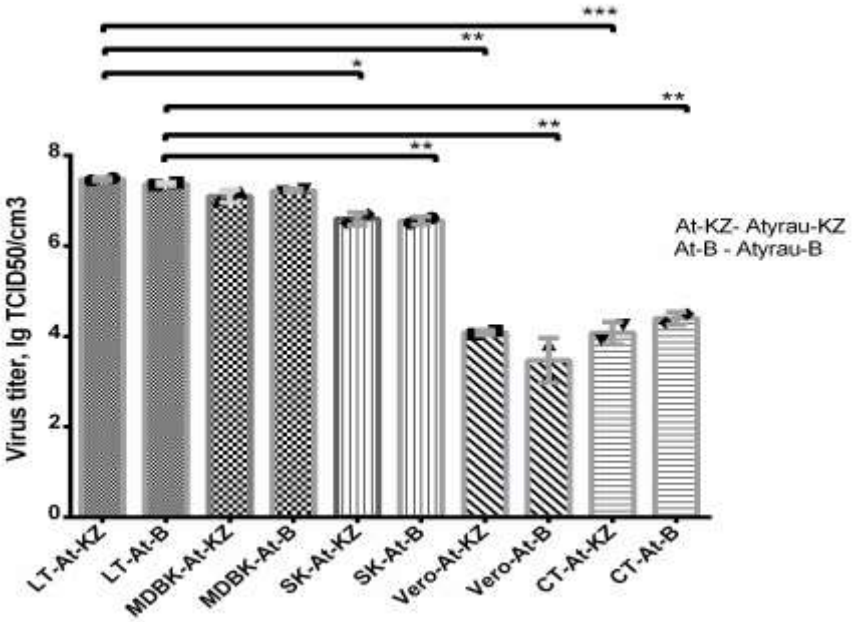


Figure 1. Infectious activity of Atyrau-KZ and Atyrau-B viruses in different cell cultures after the fifth successive passage

T a b l e

Reproduction of LSD viruses Atyrau-KZ and Atyrau-B in cell cultures (n=3)

	Atyrau-KZ			Atyrau-B		
Cell culture	**Passage number**	**Cultivation time, days**	**Infectious activity titer, lg TCD50/cm3**	**Passage number**	**Cultivation time, days**	**Infectious activity titer, lg TCD50/cm3**
LT	1	5	7,45±0,25	1	4	7,20±0,10
	2	4	7,20±0,10	2	4	7,35±0,05
	3	4	7,30±0,20	3	4	7,20±0,30
	4	4	7,80±0,10	4	4	7,00±0,10
	5	4	7,50±0,00	5	4	7,40±0,10
MDBK	1	7	7,20±0,10	1	7	7,00±0,30
	2	5	7,40±0,10	2	5	7,50±0,00
	3	5	7,30±0,40	3	5	7,10±0,00
	4	5	6,80±0,10	4	5	6,50±0,00
	5	5	7,00±0,25	5	5	6,75±0,25
SK	1	6	6,70±0,20	1	6	6,50±0,00
	2	6	6,37±0,12	2	6	6,50±0,25
	3	6	6,30±0,00	3	6	6,10±0,20
	4	6	6,75±0,00	4	6	6,50±0,00
	5	6	6,50±0,00	5	6	6,62±0,12
Vero	1	7	5,15±0,25	1	7	5,00±0,10
	2	10	4,75±0,50	2	10	4,20±,010
	3	10	4,05±0,05	3	10	3,82±0,07
	4	10	2,60±0,30	4	10	2,40±0,10
	5	10	4,12±0,87	5	10	3,12±0,37
CT	1	7	5,30±0,00	1	7	5,00±0,30
	2	7	5,20±0,10	2	7	4,70±0,20
	3	8	4,60±0,10	3	8	3,90±0,00
	4	8	4,30±0,00	4	8	3,80±0,30
	5	8	4,50±0,00	5	8	4,25±0,50

From the data presented in Table, it can be seen that the accumulation of lumpy dermatitis viruses Atyrau-B and Atyrau-KZ is not the same in the used cell cultures.

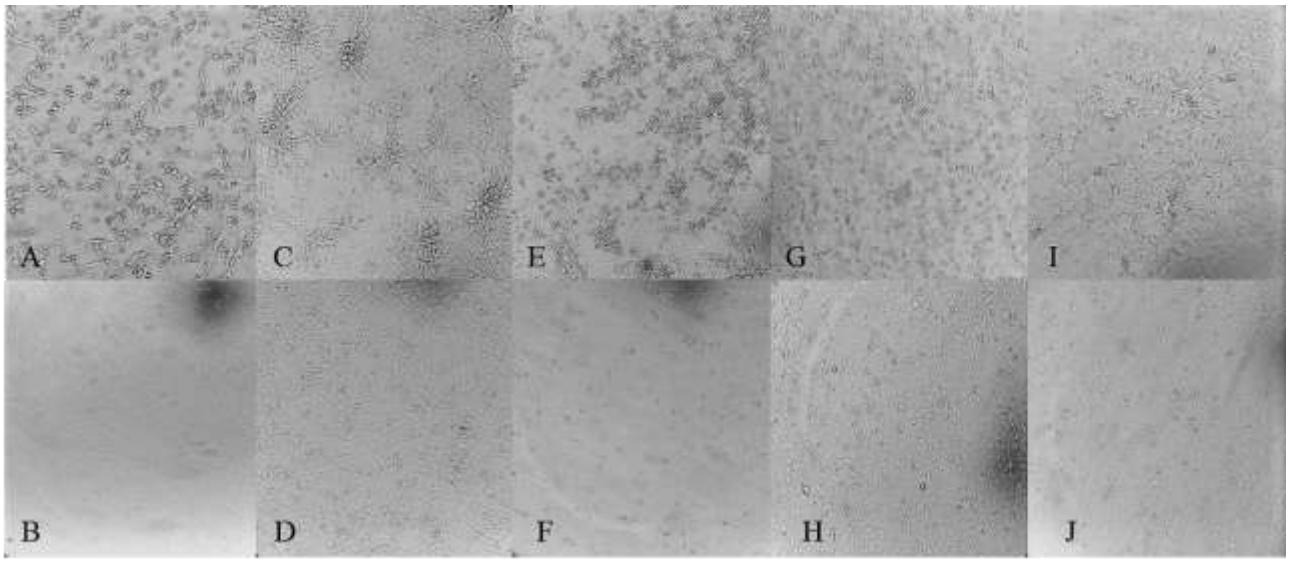
Both viruses replicated stably for five consecutive passages (observation period) in LT, MDBK, and SK cell cultures. There were no significant differences in terms of cultivation and infectious activity for Atyrau-B and Atyrau-KZ viruses. Complete damage to the monolayer of LT cells was noted after 4 days of cultivation, infectious activity reached 7.0-7.5 lg TCD50/cm^3. In MDBK and SK cells, the virus accumulated up to 6.5-7.5 and 6.0-6.5 lg TCD50/cm3, respectively. The duration of incubation was 5 and 6 days, respectively.

In cultures of Vero and CT cells, even with an increase in the cultivation time, the activity of both viruses in the process of passaging significantly decreased compared to the culture of LT cells ($p<0.001$) and ($p<0.0$ 01), respectively (Fig. 1). The titer of Atyrau-KZ and Atyrau-B viruses in Vero cells was within 2.60±0.30-5.15±0.25, 2.40±0.10-5.00±0.10 lg TCID50/cm^3, in CT cells 4.30±0.00-5.30±0.00, 3.80±0.30-5.00±0.30 lg TCID50/cm3, respectively.

Lumpy skin dermatitis viruses Atyrau-KZ and Atyrau-B exhibited a similar cytopathic effect in the monolayer of used cell cultures (Fig. 2). At the same time, the morphological manifestation of the cytopathic action of viruses differed in different cultures.

In the culture of LT and SK cells, 72 hours after infection, the cells begin to stretch and separate, by 3-4 days, fusion of cell membranes and rupture of the monolayer were observed (Fig. 2A and 2G). In MDBK cells, 72 hours after infection, the formation of pronounced conglomerates was observed, and by day 4, the monolayer was broken and fragmented (Fig. 2C).

Visible changes in Vero and CT cell cultures were noted on days 5–6 of cultivation in the form of deformation of individual cells, by days 7–8, the formation of pronounced localized foci of cell damage with the formation of conglomerates (Fig. 2E and 2I) .



A - Monolayer of LT cell culture 72 h after infection with the Atyrau-B virus; B - Monolayer of uninfected LT cell culture; C - Monolayer of MDBK cell culture 72 h after infection with Atyrau-B virus; D - Monolayer of uninfected MDBK cell culture; (E) Vero cell culture monolayer 72 h after infection with the Atyrau-B virus; F, Monolayer of uninfected Vero cell culture; G, Monolayer of SK cell culture 72 h after infection with the Atyrau-B virus; H, monolayer of uninfected SK cell culture; I – Monolayer of CT cell culture 72 h after infection with the Atyrau-B virus; J – Monolayer of uninfected CT cell culture

Figure 2. Cytopathic effect of lumpy skin disease virus Atyrau-B in the studied cell cultures (magnification 10×)

Conclusions

Currently, a large number of poxvirus genes encoding virulence factors have been identified. It has been experimentally confirmed that knockout of some of them leads to attenuation of viruses in vivo. The LSDV008 gene is a homologue of the B8R gene of the vaccinia virus. Poxvirus interferon-γ receptor-like proteins generally show limited affinity to the extracellular domains of the mammalian interferon-γ receptor and likely competitively prevent interferons from binding to their native receptors. Knocking out virulence genes to produce attenuated viruses should not affect the reproduction of viruses in vitro, since the production of vaccines requires the production of highly active viral suspensions. As a result of our studies, it was established that knockout of the LSDV008 gene did not affect the replication activity of the recombinant Atyrau-B virus in vitro. The most sensitive cell systems for reproduction of both parental Atyrau-KZ and recombinant Atyrau-B viruses are LT, MDBK, and SK cell cultures. The resulting recombinant will be used to develop a new generation of vaccines against lumpy dermatitis in cattle. The possibility of its use as a vaccine vector for the creation of polyvalent vaccines against infectious diseases of animals will also be evaluated.

Acknowledgments

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LSDV008 генінің нокаутының рекомбинантты нодулярлы дерматит вирусының өсу қасиеттеріне әсері

Нодулярлы дерматит вирусы (LSDV) — ірі қара малда ауыр жүйелі ауруды тудыратын және географикалық шекарасын жылдам кеңейтетін поксвирус. Нодулярлы дерматит (НД) безгегімен, теріде, шырышты қабаттарда және ішкі мүшелерде түйіндер пайда болуымен сипатталады. Аурудың лимфа түйіндері ісінгенде малдың арықтауына немесе өлімге әкелуі мүмкін. Соңғы жылдары бұл ауру Азияның әртүрлі бөліктерінде эндемиялық сипатқа ие болып, мал шаруашылығына айтарлықтай экономикалық зиян келтірді. НД үшін арнайы емдеу құралдары болмағандықтан, вакцинация ауруды бақылау және жоюдың ең тиімді әдісі. Толық жарамды иммунитетті тірі әлсіретілген вакциналармен вакцинациялау арқылы алуға болады. Ұзақ жүргізілген пассаждар арқылы пайда болған аттенуация геномдағы кездейсоқ мутациялармен байланысты және әлсіреу механизмі анық емес. Вирустық геномдағы вируленттілік гендерін гендік инженерия әдістері арқылы жою — жоғары әлсіреген поксвирустарды құрудағы ең перспективалы бағыт. LSDV008 гені γ-интерферон рецепторына ұқсас ақуызды кодтайды және НД вирусы үшін потенциалды вирулентті ген болып саналады. Бұл зерттеулерде біз LSDV008 генінің жойылуының Atyrau-B рекомбинантты вирусының өсу қасиеттеріне әсерін зерттедік. Бақылау ретінде ата-аналық НД вирусы Dermatitis nodulares/2016/Atyrau/KZ (Atyrau-KZ) қолданылды. Зерттеулер нәтижесінде ген нокаутының *in vitro* рекомбинантты Atyrau-B вирусының репликация белсенділігіне әсер етпейтіні анықталды. Рекомбинантты вирус жасушаларда ата-аналық вируспен бірдей титрлерде жинақталған. Atyrau-KZ және Atyrau-B НД вирустарының көбеюі үшін ең сезімтал жасушалық жүйелер — қозының аталық безі (LT), бұқа бүйрегі (MDBK) және ақбөкен бүйрегі (SK) жасушалары және де оларды әрі қарай ғылыми зерттеулерде вирустық массаны алу үшін пайдалануға болады.

Кілт сөздер: нодулярлы дерматит вирусы, жасуша өсіндісі, өсіру, вирус титрі, репликация, инфекциялық белсенділік, ген нокауты, аттенуация.

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Влияние нокаута гена LSDV008 на культуральные свойства рекомбинантного вируса нодулярного дерматита

Вирус нодулярного дерматита (LSDV) представляет собой поксвирус, который вызывает тяжелое системное заболевание крупного рогатого скота и быстро расширяет свои географические границы. Но-

дулярный дерматит (НД) характеризуется лихорадкой, образованием узелков на коже, слизистых оболочках и внутренних органах. Заболевание может вызвать исхудание с увеличением лимфатических узлов, а иногда и гибель. В последние годы данное заболевание имеет эндемичное значение в различных частях Азии, нанося значительный экономический ущерб животноводству. Так как отсутствуют специфические методы лечения НД, вакцинация является наиболее эффективным способом контроля и искоренения болезни. Наиболее полноценный иммунитет можно получить при вакцинации живыми аттенуированными вакцинами. Аттенуация посредством длительных пассажей связана со случайными мутациями в геноме, и механизм аттенуации остается не ясен. Направленное удаление генов вирулентности в вирусном геноме методами генной инженерии является наиболее перспективным направлением в создании аттенуированных поксвирусов. Ген LSDV008 кодирует белок, подобный рецептору γ-интерферона, и является потенциальным геном вирулентности вируса НД. В настоящей работе мы изучили влияние делеции гена *LSDV008* на культуральные свойства рекомбинантного вируса *Atyrau–B*. В качестве контроля использовали родительский вирус НД *Dermatitis nodulares/2016/Atyrau–KZ (Atyrau–KZ*). В результате проведенных исследований установлено, что нокаут гена не повлиял на репликационную активность рекомбинантного вируса *Atyrau–B in vitro*. Рекомбинантный вирус накапливался в культурах клеток в таких же титрах, как и родительский вирус. Наиболее чувствительными клеточными системами для репродукции вирусов *НД Atyrau–KZ* и *Atyrau–B* являются культуры клеток тестикул ягненка (LT), почки быка (MDBK) и почки сайги (SK), которые могут быть использованы для получения вирусной массы в дальнейших научных исследованиях.

Ключевые слова: вирус нодулярного дерматита, культура клеток, культивирование, титр вируса, репликация, инфекционная активность, нокаут генов, аттенуация.