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АНАЛИЗ СЛУЧАЙНОЙ ИНТЕГРАЦИИ РЕКОМБИНАНТНОГО АДЕНОАССОЦИИРОВАННОГО ВИРУСА-6, УПАКОВАННОГО В КЛЕТКИ Sf9 НАСЕКОМЫХ[1]

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В последнее время растет озабоченность по поводу интеграции в геном вектора рекомбинантного аденоассоциированного вируса (rAAV), используемого для генной терапии. Аденоассоциированный вирус дикого типа (AAV) специфически интегрируется в *AAVS1*-сайт генома человека, в то время как rAAV случайным образом интегрируется в хромосомы хозяина с низкой частотой. Проанализированы события случайной интеграции конструкции rAAV6-EGFP, упакованной в клетки Sf9 насекомых. Производственная платформа Baculo-Sf9 обладает преимуществами суспензионной культуры клеток насекомых Sf9: высокой плотностью и возможностью крупномасштабного производства векторов rAAV. В проведенном исследовании использованы различные дозы вектора rAAV6-EGFP, продуцируемого Baculo-Sf9, для трансдукции клеток HEK293T и A549-имплантированных опухолей *in vitro* и *in vivo*. Методами проточной цитометрии и флуоресцентной микроскопии оценена эффективность экспрессии гена *egfp* и интенсивность флуоресценции EGFP. С помощью инвертированной гнездовой ПЦР и секвенирования ДНК идентифицированы случайные сайты интеграции генома rAAV6-EGFP в хромосомы человека. По результатам анализа *in vitro* показано, что эффективность экспрессии репортерной конструкции стабилизировалась через 20 суток, а частота случайной интеграции составляла 0.2–4.2%. В исследованиях как *in vitro*, так и *in vivo* выявлено, что случайная интеграция rAAV6 зависела от дозы конструкта. По результатам секвенирования идентифицировано два случайных сайта интеграции, которые находятся на хромосомах человека 8 и 12. На основании полученных данных можно сделать вывод, что для безопасной генной терапии следует использовать как можно более низкие дозы вектора rAAV.

Ключевые слова: рекомбинантный аденоассоциированный вирус, бакуловирусная система Sf9, случайная интеграция, инвертированная гнездовая ПЦР

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Random Integration Analysis of Recombinant Adeno-Associated Virus 6 Packaged in Sf9 Insect Cells

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Recently, there have been growing concerns over the integration of recombinant adeno-associated virus (rAAV) used in gene therapy. Wild-type adeno-associated virus **(**AAV) site specifically integrates into *AAVS1* site of human genome, while rAAV randomly integrates into host chromosomes at low frequencies. This research

aims to study the random integration events of rAAV6-EGFP packaged in Sf9 insect cells. Baculo-Sf9 manufacturing platform has the advantages of high-density suspension culture of Sf9 insect cells and large-scale production of rAAV vectors. In this study, we used different doses of Baculo-Sf9 produced rAAV6-EGFP to transduce HEK293T cells and A549-implanted tumors *in vitro* and *in vivo*. Using flow cytometry and fluorescence microscopy, we studied their *EGFP* gene expression efficiencies and EGFP fluorescence intensities. Using inverse nested PCR and DNA sequencing, random integration sites of rAAV6-EGFP genome into human chromosomes were identified. *In vitro* results showed that gene expression efficiencies became stable after 20 days and random integration frequencies were 0.2–4.2%. Both *in vitro* and *in vivo* results indicated that random integration of Baculo-Sf9 rAAV6 was dose-dependent. Sequencing results showed two random integration sites, which were on human chromosomes 8 and 12. The findings suggest that we should use as low dose of rAAV vector as possible for safe gene therapy.

Keywords: recombinant adeno-associated virus, Baculo-Sf9 manufacturing platform, random integration, inverse nested PCR