**Construction and immunoefficacy evaluation of a live attenuated influenza A H1N1 vaccine candidate based on M gene modification**

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**Abstract:**

**Background/Objective:** Influenza virus vaccination is the most effective strategy against influenza outbreaks and seasonal epidemics at present. It was reported that the development influenza attenuated strains by the gene modification of IAV M segment is one of the feasible options for the research of live attenuated influenza vaccines.

**Methods:** Based on the IAV strain (A/Puerto Rico/8/1934, PR8) as the skeleton, the synonymous mutations were carried out in the M1 and M2 overlapping regions of the M gene of PR8 strain to ensure the integrity of the amino acid sequence of M1 and the multiple amino acid mutations of M2, and meanwhile the deletion mutations of M2 were carried out to construct the reverse genetic operating system of PR8. The HA and NA genes of H1N1 subtype IV（A/Victoria/2570/2019, H1N1/2019）, a trivalent influenza vaccine circulating in the Northern Hemisphere recommended by WHO for the chicken embryo production during 2022 to 2023, were recombined with the other 6 internal genes of PR8, and then were transfected with the expression protein granules into the 293T cells to save A H1N1 influenza virus strain.

**Results:** Compared with the original strain, the rescued virus strain showed the limited growth in MDCK cells，but showed the better growth and good passage stability in MDCK-M2 cells, no revert mutation occurred after multiple passage. It was not strong dependence in cell lines and could grow normally in the chicken embryos. The verifying results based on Balb/C mouse model showed that the attenuated strain had decreased toxicity compared with the original strain, and there was non-lethal to mice with high safety.

**Conclusion:** In this study, A attenuated influenza A H1N1 virus strain was successfully constructed, which had obvious attenuated characteristics, and produced good safety, immunogenicity and virus-challenging protection effect after immunizing mice.

**Keywords:** Influenza A virus; M gene; Reverse genetics; Attenuated live vaccine; Immune efficacy evaluation