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Production of Immortalized Fetal Kidney Lamb Cell Lines for Veterinary Biotechnology Applications

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Abstract

Primary kidney cells are traditionally used for propagation of live-attenuated vaccines: sheep-poxvirus or capri-poxviruses, but require iterative preparations of primary cells isolated from fetal animal kidneys. Sheep primary epithelial cells are short-lived in cell culture systems. The culture of kidney-derived primary epithelial cells is limited to a very low number (5-6) of passages so that they require frequent replenishment for their use which is associated with multiple risks (genetic variability of donors, infections by pathogens, fragility during culture, etc.). To overcome these constraints, the immortalization of these cells in permanent cell lines, constitutes a long-term approach. Here we describe the immortalization of primary fetal kidney lamb cells using a plasmid expressing the sequence encoding the SV40 large T-antigen. The resulting immortalized cells were characterized and manifested many important properties in cultures.

The success of the primary cell transfection and cell cloning stages allowed to pursue on the characterization of the cloned cell lines by studying cell morphology, their multiplication capacity in culture, their cytogenetic properties, as well as the biochemical and molecular characteristics. After about twenty passages, the immortalized cells retained morphological aspects similar to that of the parental primary epithelial cells and the growth properties became completely different. The polyclonals TISFKC as well as the TISFKC clones showed a greater capacity for proliferation in culture compared to the parental primary cells. Even if, in the early stages of immortalization, the cells showed a heterogeneous polyploidy, the karyotype gradually became stable, identical to that of the primary cells SFKC (2n=54). Immunocytochemistry detection of SV40 T expression was successful on both the polyclonal TISEKC and those of 20 clonal cell lines. Interestingly cells of the clone TISFKC-5 were found to be negative for SV40 T antigen expression, although they maintain all the other characteristics similar to the polyclonal and 20 other clones . PCR analysis showed clearly the presence of SV40 T antigen gene in total DNA from TISEKC-5 as well as crude TISEKC. Epithelial phenotype was well conserved in all cloned anfd polyclonal cellc as revealed by Immunodetection of cytokeratin and vimentin protein expression. The fact that TISFKC-5 cell line expresses no longer the SV40 T oncogene, its normal karyotype and the fact

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that the immortalized TISEKC cells could effectively be transfected with plasmids expressing any proteins of interest, makes this cell line a valuable tool for fundamental studies and for biotechnological development of veterinary products. In parallel, the TISEKCs had subsequently undergone several passages in culture and several cycles of freezing and storage in liquid nitrogen, then thawing and re-cultured without showing any alteration of their viability or growth capacity. Thus, immortalized cell lines offer several advantages such as rapid cell availability at any time, capacity for wide expansion, unlimited cell division power and having a very long lifetime. They are cost effective, easy to use, provide an unlimited supply of material, and circumvent the ethical concerns associated with the use of animal and human tissues. This cell line offers the possibility of veterinary vaccine production in similar conditions used for human vaccines as required by the One-health concept.

Keywords:
Epithelial kidney cells; SV40 T antigen; Transfection; Cell immortalization.

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