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Development, characterization and optimization of a new suspension chicken-induced pluripotent cell line for the production of Newcastle disease vaccine

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ABSTRACT

Traditionally, substrates for production of viral poultry vaccines have been embryonated eggs or adherent primary cell cultures. The difficulties and cost involved in scaling up these substrates in cases of increased demand have been a limitation for vaccine production. Here, we assess the ability of a newly developed chicken-induced pluripotent cell line, BA3, to support replication and growth of Newcastle disease virus (NDV) LaSota vaccine strain. The characteristics and growth profile of the cells were also investigated. BA3 cells could grow in suspension in different media to a high density of up to 7.0×10^6 cells/mL and showed rapid proliferation with doubling time of 21 h. Upon infection, a high virus titer of 1.02×10^8 EID_{50}/mL was obtained at 24 h post infection using a multiplicity of infection (MOI) of 5. In addition, the cell line was shown to be free of endogenous and exogenous Avian Leukosis viruses, Reticuloendotheliosis virus, Fowl Adenovirus, Marek's disease virus, and several Mycoplasma species. In conclusion, BA3 cell line is potentially an excellent candidate for vaccine production due to its highly desirable industrially friendly characteristics of growing to high cell density and capability of growth in serum free medium.

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1. Introduction

Newcastle disease (ND) is a major poultry disease causing significant economic losses and is a major threat to food security in many countries [1]. It is the most devastating disease for rural poultry, especially in the developing world [2]. The disease has been reported in most bird species with chickens being the most susceptible and the disease may range from mild, with little or no apparent clinical signs, to severe with 100% mortality [3].

Vaccination is the most accepted prevention and control strategy for combating ND in poultry worldwide. Commonly used vaccines are made with low virulence viruses, which provide both cellular and humoral immunity at a low cost, and inactivated oil-emulsion of the same viruses which confer a higher and long lasting humoral immunity [4–6]. In addition different recombinant NDV vaccines based on low virulence avian viruses (Herpesvirus of Turkey, Fowlpox or low virulence NDV) have been developed and

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used with great success experimentally. Recombinant vaccines are generated by cloning the fusion (F) and hemagglutinin-neuraminidase (HN) gene of circulating genotype into a viral backbone this thereby provides protection against clinical disease and shedding of virulent challenge virus [7–10] Different vaccines are commercially available and live ND vaccines are largely produced in specific pathogen free (SPF) chicken embryonating eggs. The production of SPF eggs requires significant resources for the procurement and maintenance of a disease free rearing facility; hence there is a limited number of facilities globally producing SPF eggs [11]. In times of high demand other vaccine manufacturers (e.g. avian influenza) import SPF eggs, thus creating challenges for availability, logistics, and costs. The high dependence of vaccine production on perishable SPF embryonating eggs, loss of birds to disease, or antibody development in surviving birds are factors that often renders flock and eggs unusable for vaccine production and may stall manufacturing. These limitations are accentuated in the developing world where SPF eggs are expensive and not always available to vaccine producers during outbreaks.

MDCK and Vero cell lines have been extensively studied and used by manufacturers for the production of approved influenza virus vaccine [12–14]. Unlike influenza virus vaccine production, limited avian cell lines have been explored for NDV vaccine production [15,16] hence manufacturing of NDV vaccine largely depends on embryonated eggs for production. Some primary and continuous cell lines of avian and non-avian origin have been used in the propagation of NDV. These include chick embryo fibroblast (CEF), chicken embryo liver (CEL) cells, chicken embryo kidney (CEK) cells, African green monkey kidney (Vero) cells, and DF-1 (a cell line derived from CEF) cells [16,17]. Primary cells have the disadvantage of senescence leading to the need for regular and costly re-derivation and characterization and the fact that they may not always comply with modern quality standards required for good manufacturing practices. These cells are anchorage dependent and not always easily amenable to scale up for large scale vaccine production, which is often done in suspension to maximize space and cell density [18]. Use of suspension culture makes vaccine production easier because there is no need for trypsinization or anchorage support for the cells. This has created significant interest in developing immortal and stable cell lines with the capability of growth in suspension. Additionally, it is also of importance to be able to expand cells in serum free medium for vaccine production as it eliminates a potential contamination (e.g. prion, viral) source and significantly reduces production cost, which is critical as most chicken vaccines must be economical to be cost effective for producers. It is also of significant interest to produce vaccine virus in cells from the target host species (*Gallus gallus*) to prevent mutations and reversion to virulence that may make it less effective as a vaccine.

In recent studies, researchers have succeeded in growing adherent cells like Vero and MDCK on micro-carriers [19,20] or adapting them to grow in suspension [21,22] for Influenza vaccine production. In other attempts, “designer” cell lines like human PER.C6 [23], EB66 [24], AGE1.CR.pIX [25], and CAP [26] with industrial friendly properties have been developed and used for vaccine production. In order to add to the choice of cell lines available for vaccine production a new cell line derived from non-viral minicircle DNA method of reprogramming was developed [27]. This process of cell reprogramming has received the approval of the Food and Drug Administration (FDA) and is being used widely for research and development [28].

This study focuses on describing, for the first time, the characteristics of a newly developed serum-free, suspension chicken-induced pluripotent cell (SciPC) line BA3 and determining the potential of this cell line as an alternative to egg based ND virus vaccine production. The BA3 cell line was tested and found to be free of endogenous and exogenous avian retroviruses as well as other adventitious agents. This makes it a potential candidate for the production of animal and human vaccines on an industrial scale.

2. Materials and methods

2.1. Cell line

The BA3 ciPC line was derived as previously described by Lu et al. [29]. Briefly, day 11 chicken embryos were used to isolate for chicken embryonic fibroblasts (CEFs). CEFs were cultured in fibroblast medium (DMEM high glucose (Hyclone) with 10%FBS (Hyclone), 4 mM L-Glutamine (Gibco) and 50 U/mL penicillin and 50 μg/mL streptomycin (Gibco)) in 5% CO_2 at 37 °C, and split using 0.05% trypsin (Gibco) as they reached to confluence. For transduction, a total of 150,000 CEFs were plated in one well of a 12-well plate. After 24 h, CEFs underwent lentiviral transduction utilizing the viPC kit (Thermo Scientific) with viruses containing the human stem cell genes POU5F1, NANOG, SOX2, LIN28, KLF4 and C-MYC under the promoter of human elongation factor-1 alpha (EF1a) (EF1 underwent lentiviral agents). CEFs were trypsinized 24 h after transduction and passaged onto inactivated feeder cells in embryonic stem cell expansion medium (Dulbecco's modified Eagle medium (DMEM)/F12 (Gibco), supplemented with 20% knockout serum replacement (KSR; Gibco), 2 mM L-Glutamine (Gibco), 0.1 mM non-essential amino acids (Gibco), 50 U/mL penicillin, 50 μg/mL streptomycin (Gibco), 0.1 mM mercaptoethanol (Sigma–Aldrich) and 10 ng/mL basic fibroblast growth factor (bFGF; Sigma–Aldrich and R&D System)). ciPC-like cells were manually harvested and plated on Matrigel (BD Biosciences; diluted 1:100 in DMEF/F12) coated dishes in mTeSR1 medium (Stemcell Technologies). ciPC-like cells were mechanically dissociated using a glass Pasteur pipette every 4–5 days. Cells were then transferred into 20% KSR embryonic stem cell expansion medium, while still plated on Matrigel. Cells adjusted to the 20% KSR medium and were passaged using 0.05% trypsin once reaching 85% confluence.

2.1.1. Adaptation of BA3 cell line to serum free medium and suspension growth

A step-wise reduction of the percentage of KSR medium (20–1%) was performed to determine optimum concentration for effective cell growth. The resulting cells, named suspension ciPC (SciPC) BA3 cells, from the reduced KSR treatment were grown in a petri dish on a shaker agitated at 50–100 rpm in 5% CO_2 atmosphere at 37 °C. The bFGF from the medium was equally removed.

2.2. Characterization of the suspension ciPC line

2.2.1. Growth profile (saturation density and doubling time)

The SciPC cells were grown in DMEM/F12 with and without 15 mM HEPES supplemented with 5% KSR and seeding density of 7.0×10^5 cells/mL with continuous agitation on a shaker at 50–100 rpm in 5% CO_2 atmosphere at 37 °C with medium changes every 24 h. Cell counting was performed using Cellometer Auto T4 (Nexcelom Bioscience LLC, Lawrence, MA) with 0.4% trypan blue.

2.2.2. Testing for the presence of avian leucosis, reticuloendotheliosis viruses, Fowl adenovirus, Marek's disease virus, and mycoplasma

One of the major limitations of establishing new cell lines is the presence of congenitally transmitted as well as endogenous viruses, making them unsuitable for vaccine production. Media samples from the BA3 cell line were inoculated onto chicken embryo