Characterization of GSF289: a fibroblast cell line derived from goat ear skin explants

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Recently we established three fibroblast cell lines from ear skin explants of normal healthy dairy goats, of Kiko and Saanen breed (*In Vitro Cell.Dev.Biol.-Animal; doi: 10.1007/s11626-010-9373-4*). The cell lines revealed a viability rate of 96.2%, displayed a typical 'S' shaped growth curve with a population doubling time of 25 hours, and were free from microbial, fungal and mycoplasma contamination. We further characterized these cell lines and, in this communication we show the cytogenetic analysis and the genetic transfection of GSF289 cells. The GSF289 cell line which originated from Saanen breed of goats was successfully transfected with pcDNA3.1/NT-GFP plasmid vector containing green fluorescent protein (GFP) gene under human cytomegalovirus (CMV) promoter. The efficiency of transfection, as measured by flow cytometry, was 14.5% after 4 days of culture. The cytogenetic analysis performed on 29 G-banded metaphase cells revealed that the cell line has a normal male goat karyotype consisting of 58 autosomes and two XY sex chromosomes. These results suggest that GSF289 cell line with a normal karyotype, having a high rate of proliferation, and its ability to be easily transfected with plasmid DNA vectors is an additional tool to study molecular mechanisms that regulate fibroblast function as well as genetic manipulation of small ruminants.

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Introduction

Fibroblast cells have been cultured in vitro from several species [1-4]. These cultured cells have been used for in-vitro analysis of fibroblast growth, migration and collagen metabolism [5], to study the pathophysiological processes [6], and to diagnose inborn errors of metabolism and pathophysiology of fibrotic skin diseases in humans [7]. Fibroblasts play a crucial role in wound healing and thus have been extensively studied to develop biomaterial implants that secrete growth factors for treatment of diabetic foot ulcers [8, 9]. Fibroblasts grown in a biodegradable mesh have been used as a living dermal replacement [10, 11] and play a key role in plastic surgery. Tissue engineered biological dressings offer a great promise in the treatment of burns, chronic ulcers, and a variety of blistering and other dermatologic conditions [12, 13]. They are a potential model to study induction of melanoma by natural sun light [14]. Additionally, they have been successfully used as feeder layers [15], for animal cloning [16, 17], and for the induction of pluripotent stem (iPS) cells [18]. We have recently established three fibroblast cell lines from goat ear skin explants [19] with an excellent viability and proliferation

rate. We further characterized these cell lines and here we show the cytogenetic analysis and the potential of these cells for genetic manipulation.

Materials and Methods

Growth media and cell culture

Porcine fibroblast complete growth medium (P-116; cat. no: P116-500) was obtained from Cell Applications, Inc. (San Diego, CA). Liquid nitrogen stocks of GSF289 cell line were expanded in T-25 tissue culture flasks. Briefly, the cryovial was thawed quickly in a 37°C water bath and the contents of the vial transferred to a 15 ml centrifuge tube containing 7 ml of warm medium. The cells were centrifuged at 200 x g for 7 min and the pellet was re-suspended in 4 ml of media. An aliquot was taken for determining the cell count by trypan blue dye exclusion method [20] and the rest of the cells were transferred to T-25 flasks for culturing at 37°C, 5% CO_2 in a humidified environment. The cells were passaged at 60-80% confluence as described before [19], and used for karyotype and transfection experiments.

Karyotyping

Goat cells at passage 4 were processed for karyotyping using previously established methodologies [21] by Cell Line Genetics (Madison, WI; www.clgenetics.com). Chromosome assignments were made according to the Atlas of Mammalian Cytogenetics [22].

Green fluorescent protein (GFP) transfection and flow cytometric analysis of GFP expression

Goat skin fibroblast (GSF289) cells were transiently transfected with pcDNA3.1/NT-GFP plasmid DNA using Lipofectamine 2000 reagent (Life Technologies Corp., Carlsbad, CA, USA) according to the manufacturer's specifications. Briefly, 24 h before the transfection, GSF289 cells were seeded in a 6-well tissue culture plate at a density of 5 x 10^5 cells in 2 ml of growth medium and incubated overnight to reach approximately 60-80% confluence. Prior to transfection, the growth medium was replaced with 2 ml of OPTI-MEM transfection medium. On the day of transfection, 2 µg of plasmid DNA was diluted into OPTI-MEM (Life Technologies Corp., Carlsbad, CA, USA) and mixed with 6 µg of Lipofectamine 2000 reagent. The DNA-Lipofectamine complexes were added to the cells and incubated for 6 h. Subsequently, the transfection medium was replaced with growth medium. The GFP expression was assessed 24-96 h post-transfection and results recorded after 96 h. An Accuri C6 Flow Cytometer System with CFlow® Plus Software was used for flowcytometric analysis. Single cell suspensions of GSF289 cells were passed through a 488 nm laser for excitation, and fluorescence emissions were collected at 530 nm for GFP expression. At least, 15,000 gated events were collected for each sample. The mock transfected cells were used to set the gate.

Results

Cytogenetic analysis of GSF289 cell line

The cytogenetic analysis of GSF289 cell line, which was derived from a male goat of Saanen breed, was performed on twenty-nine G-banded metaphase cells at passage 4. The diploid (2x) number of the chromosomes in this cell line was determined to be 60,XY. As can be seen in fig. 1 (panel B), it consisted of 58 autosomes and two (X and Y) sex chromosomes, which is consistent with earlier studies on goat cytogenetics [3, 23, 24]. In this study, out of a

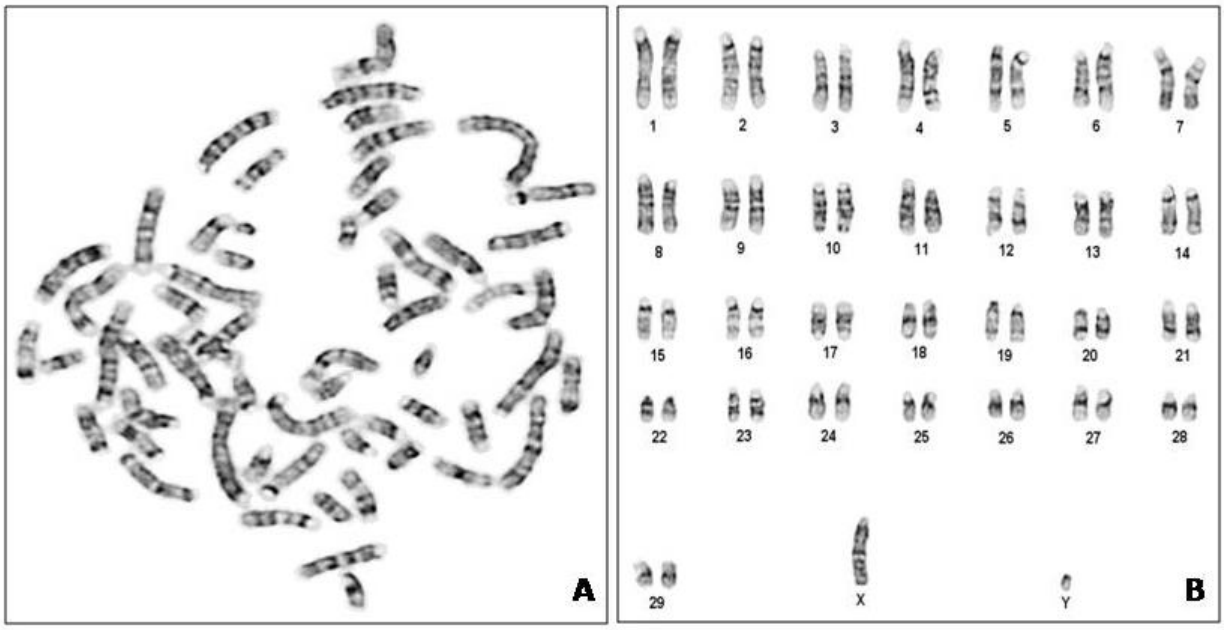

Figure 1: Karyotype of GSF289 fibroblast cells (passage 4). Panel A is a metaphase spread and panel B is karyotype.

total of 29 cells analyzed, 27 demonstrated an apparently normal male karyotype, while two cells demonstrated non-clonal chromosome aberrations. These aberrations were thought to be most likely technical artifacts and thus these results were apparently consistent with a normal male goat karyotype.

Transfection of GSF289 cells with a GFP-based plasmid DNA vector

Plasmid pcDNA3.1/NT-GFP vector containing GFP gene under human cytomegalovirus (CMV) promoter was transfected in fifth passage fibroblast cells. The expression of GFP gene was observed under Zeiss Axiovert 40 CFL Inverted microscope (Carl Zeiss) after 24-96 h post-transfection. As can be seen in fig. 2, panel B and D, the expression of GFP protein in transfected fibroblast cells was clearly visible as green fluorescence. We also performed quantitative analysis of the GFP expressing fibroblast cells using flow cytometry. The efficiency of GFP transfection was calculated as percentage of total cells expressing green fluorescence. As can be seen in fig. 2, lower panel E1-E3, 14.5% of the transfected cells in our experiments expressed GFP gene.

Discussion

Dermis, an inner thick layer of skin, is primarily composed of fibroblast cells. These cells synthesize a collagen rich extracellular matrix that provide main structure and strength to the skin. Skin fibroblast cells have been cultured in vitro from human and other animal species and have been used for both basic and applied research. In vitro culture of fibroblast cells provide an excellent model system to study the molecular mechanisms regulating fibroblast function in normal and disease tissues. In addition, in vitro culture of these cells offer great promise for producing site-specific genetically modified animals such as knock-out animals by somatic cell cloning. However, for the success of such studies, establishment of species, tissue or disease-specific fibroblast cell lines with high rate of proliferation, longevity,

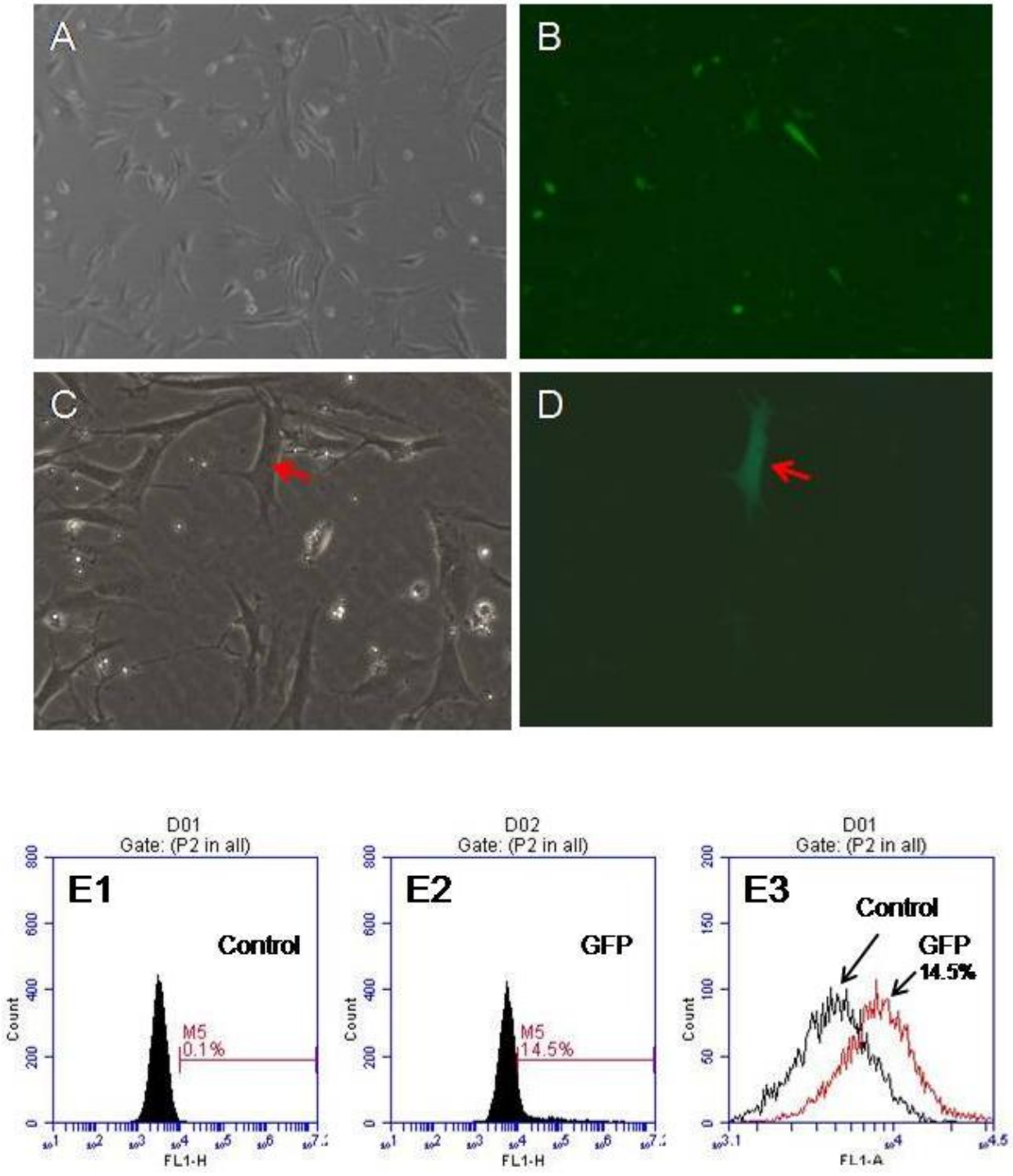

Figure 2: Transfection of fibroblast cells with GFP gene containing plasmid pcDNA3.1/NT-GFP: Panel A and B are 10x magnified light and fluorescent microscopic images of GFP transfected cells, whereas panel C and D are similar but 40x magnified images. Arrow indicates an example of a GFP transfected fibroblast cell. Lower panels E1, E2 and E3 are flow cytometric analysis of non-transfected control and GFP-transfected fibroblast cells after 4 days of transfection. The GFP expression in the fibroblast cells was visualized on a Zeiss Axiovert 40 CFL inverted microscope (Carl Zeiss). GFP fluorescence was detected using a filter set with band pass at 450 to 490 nm for excitation and band pass at 515 nm for emission.

genetic stability, and the potential for in vitro genetic manipulation are required. We had recently established three fibroblast cell lines from lower edge ear skin samples of healthy dairy goats with high rate of proliferation with a population-doubling time of 25 h and an excellent cell viability rate of 96.2% [19]. We further characterized GSF289 cell line (that was derived from Saanen breed of goats) with respect to its chromosomal profile, and its ability to gene transfection. The results demonstrated that the GSF289 cell line caries a normal male goat karyotype and it is genetically stable. Additionally, GSF289 cell line was easily transfected with a GFP gene containing plasmid DNA vector. Although the transfection efficiency in our experiments was low, increasing the transfection efficiency by standardizing various parameters and/or by testing different reagent/kit combinations from various vendors is always possible. It is easier to manipulate the genes in plasmid based DNA vectors (such as used in this study) having dual origins of replication (ori) sequences that allow these vectors to be replicated in both *E. coli* and eukaryotic cells. In conclusion, the high rate of proliferation, in combination with the ability of these GSF289 cells to be transfected with GFP plasmid, along with its normal karyotype, provide an additional tool to study molecular mechanisms regulating fibroblast function. This goat-specific fibroblast cell line can be used as an alternate animal model for human or goat-specific applications. In addition, this cell line has potential to study site-specific genetic modification of goat genome, prior to its nuclear transfer, to produce knock-out goats as has been shown earlier for goats and cattle [17, 25, 26].

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