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Beneficial effect of *30Kc6* gene expression on production of recombinant interferon-β in serum-free suspension culture of CHO cells

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ABSTRACT

The *Bombyx mori 30Kc* gene is known to have anti-apoptotic activity and can enhance the cell growth and expression of recombinant proteins in anchorage-dependent CHO cell cultures. In this study, an interferon-β (IFN-β)-producing CHO cell line, which expresses the recombinant *30Kc6* gene, was constructed to investigate the effect of *30Kc6* expression on the production of IFN-β in serum-free suspension culture. The *30Kc6* expressing cell line showed lower apoptotic activity and prolonged cell viability under apoptotic conditions induced by the addition of sodium butyrate, staurosporine, or the removal of serum. The *30Kc6* expressing cell line also suppressed the loss of mitochondrial membrane potential induced under these conditions. It was observed that viability, and production of IFN-β were also enhanced by *30Kc6* expression in serum-free suspension cultures. These results indicate that the *30Kc6* gene can positively affect the viability and production of recombinant therapeutic proteins in serum-free suspension cultures of CHO cell lines.

1. Introduction

The Chinese hamster ovary (CHO) cell is one of the most widely used mammalian cells for the production of recombinant therapeutic proteins because it can perform N-linked glycosylation and synthesizes glycan structures similar to those occurring in human proteins [1]. Optimization of mammalian cell culture is essential for the economical production of recombinant therapeutic proteins [2,3]. A major problem encountered during cell culture is cell death, which can decrease protein production and yield. Programmed cell death (apoptosis) can be triggered by nutrient depletion, hypoxia, waste by-product accumulation, and other factors encountered during cell culture.

There have been many efforts to inhibit the cellular apoptosis of CHO cell lines. One strategy manipulates the culture environment with component supplementations. Addition of suramin [4], insulin-like growth factor-1, transferrin [5], and some amino acids [6] have been shown to inhibit apoptosis in culture. The addition of caspase inhibitors has also been shown to suppress apoptosis and prolong cell viability [7,8]. Another approach used for the inhibition of apoptosis is the alteration of intracellular physiology by genetic engineering. There are several cellular proteins that have been shown to inhibit apoptosis and their expression can delay apoptosis in cell culture.

Bcl-2 and Bcl-x_L are prominent anti-apoptotic proteins that inhibit the release of pro-apoptotic proteins from the mitochondria. When mammalian cells are transfected with *bcl-2* they show higher cell viability during adverse culture conditions [9–11]. The expression of *bcl-*x_L is another effective method used to inhibit cellular apoptosis in mammalian cells [12–14]. The X-linked inhibitor of apoptosis (XIAP) and the cytokine response modifier (CrmA), both of which inhibit caspases, can also be expressed within mammalian cells to promote cell viability [15]. All these methods aim to increase cell survival and maximize the expression of recombinant proteins in mammalian cells.

It was previously reported that silkworm hemolymph (SH) inhibits apoptosis [16–20] and that the SH 30K proteins have apoptosis-inhibiting activities [21]. The *30Kc6* gene, a member of the sub-family of *Bombyx mori 30K* genes, can inhibit cellular apoptosis in HEK293 and CHO-K1 cells, and its anti-apoptotic effect is comparable to when SH was added to the culture medium [22]. The *30Kc6* gene also inhibits serum-deprivation induced apoptosis, increases cell density, and increase EPO production in anchorage-dependent cultures of recombinant CHO cells [23]. There has been no further study on the effect of *30Kc6* expression in serum-free suspension cultures.

Serum-free media have been used for the production of recombinant proteins in mammalian cell culture because serum

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brings regulatory issues due to the potential presence of adventitious viruses, and also complicates downstream processing. However, serum deprivation can also induce cellular apoptosis. So, construction of an anti-apoptotic cell line is needed to maximize the production of recombinant proteins in serum-free medium. Suspension cultures of mammalian cells have been widely employed in recombinant protein production because of its ease and scalability in comparison with anchorage-dependent cultures. In this study, the *30Kc6* gene was transfected into an IFN-β producing CHO cell line to investigate its effect on apoptosis and

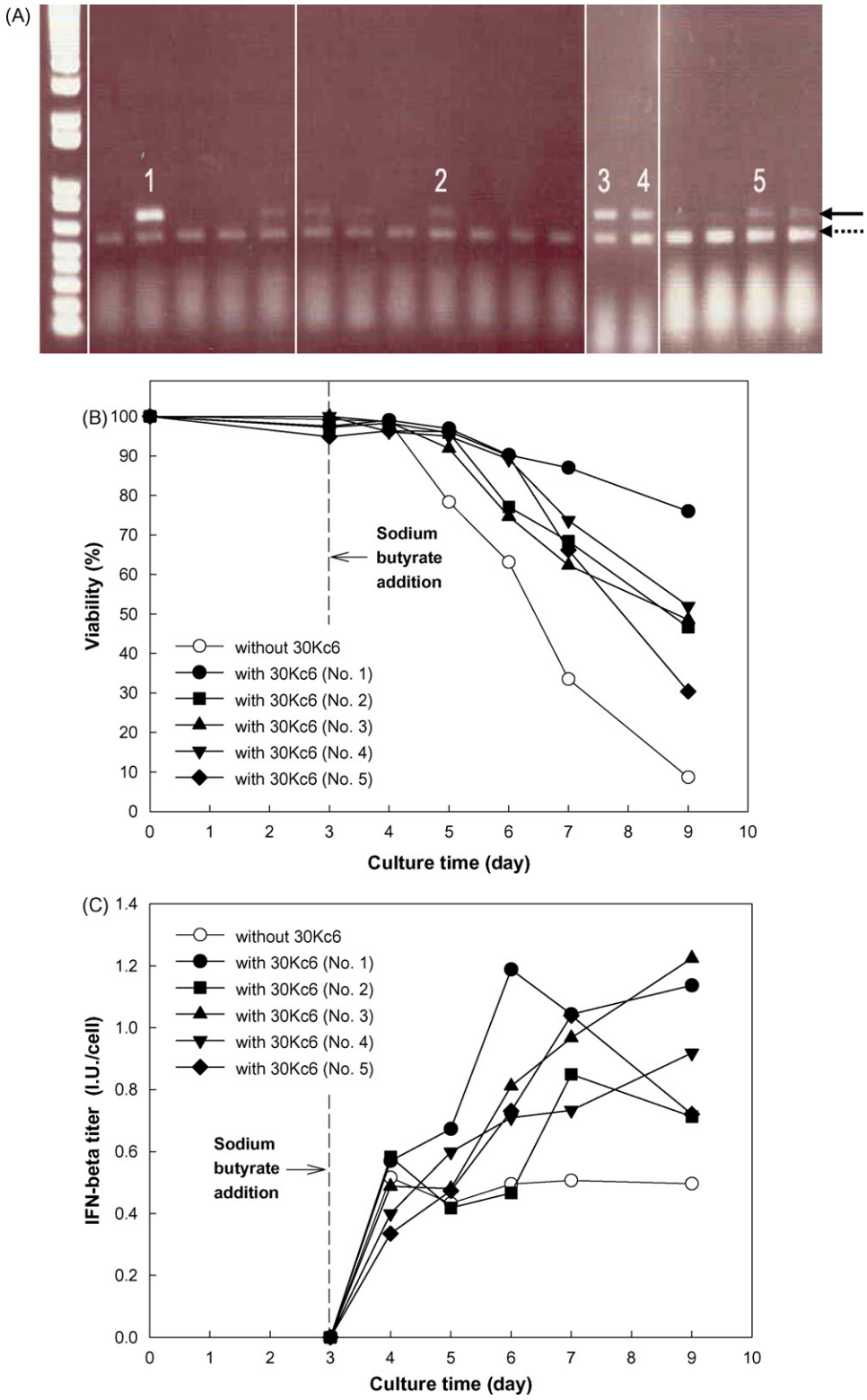

Fig. 1. RT-PCR result of *30Kc6* transfected CHO cell lines (A). The solid arrow indicates the *30Kc6* cDNA and the dotted arrow indicates CHO β-actin cDNA amplified by RT-PCR. Numbers correspond to the number of 5 selected cell lines which showed over-expression of *30Kc6*. The viability (B) and IFN-β titer per unit cell (C) of the CHO cell lines expressing recombinant IFN-β, with and without the *30Kc6* gene, when grown in anchorage-dependent cultures under apoptosis-induced condition.