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Rapid Establishment of CHO Cell Lines Producing the Anti-Hepatocyte Growth Factor Antibody SFN68

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Anti-hepatocyte growth factor (anti-HGF) monoclonal antibodies (mAbs) are potential therapeutics against various cancers. Screening for high-producer clones is a time-consuming and complex process and is a major hurdle in the development of therapeutic mAbs. Here, we describe an efficient approach that allows the selection of high-producer Chinese hamster ovary (CHO) cell lines producing the novel anti-HGF mAb SFN68, which was generated previously by immunizing HGF bound to its receptor c-Met. We selected an SFN68-producing parental cell line *via* transfection of the dihydrofolate reductase-deficient CHO cell line DG44, which was preadapted to serum-free suspension culture, with an SFN68-expression vector. Subsequent gene amplification *via* multiple passages of the parental cell line in a methotrexate-containing medium over 4 weeks, followed by clonal isolation, enabled us to isolate two cell lines, 2F7 and 2H4, with 3-fold higher specific productivity. We also screened 72 different media formulated with diverse feed and basal media to develop a suboptimized medium. In the established suboptimized medium, the highest anti-HGF mAb yields of the 2F7 and 2H4 clones were 842 and 861 mg/l, respectively, which were about 10.5-fold higher than that of the parental cell line in a non-optimized basal medium. The selected CHO cell lines secreting high titers of SFN68 would be useful for the production of sufficient amounts of antibodies for efficacy evaluation in preclinical and early clinical studies.

Keywords: Chinese hamster ovary cells, anti-hepatocyte growth factor antibody, therapeutic antibody, anticancer agents, HGF/c-Met signaling pathway, gene amplification

Introduction

Hepatocyte growth factor (HGF) plays important roles in cell proliferation, survival, motility, migration, and angiogenesis [2]. Recent studies have shown that abnormal signaling through the only known receptor of HGF, c-Met, with its tyrosine kinase activity, is associated with many types of cancers, and elevated levels of circulating HGF were shown to be correlated with tumor metastasis and cancer progression [21, 24]. These previous findings have led to the development of specific inhibitors of the HGF-c-Met pathway as novel therapeutics for various cancers [6, 10, 20]. Small-molecule c-Met tyrosine kinase inhibitors or antibodies directed against c-Met or HGF showed promising therapeutic efficacy in preclinical and clinical trials [7, 13, 16, 18]. Thus, inhibition of HGF/c-Met pathways has emerged as a new paradigm of therapy for the treatment of various types of cancers.

The production of large amounts of antibody is a large burden, particularly when evaluating the *in vivo* therapeutic potency of multiple antibody leads, because most therapeutic antibodies require high-dose administration, ranging from 5 to 10 mg/kg, over a long period in preclinical and clinical studies. In addition, it is of great importance in the

pharmaceutical industry to shorten the lengthy screening processes for isolating cell lines producing high titers of antibodies with acceptable product quality profiles. For the production of large amounts of antibody in a limited timeline, it is certainly critical to establish a simple approach for cell line establishment and media optimization. For high-level expression of monoclonal antibodies, CHO cells have proven to be most effective [4]. The establishment of high-production CHO cell lines is initiated by the transfection of an expression vector harboring the target gene and the gene encoding dihydrofolate reductase (DHFR) into DHFR-deficient cells for gene amplification using methotrexate (MTX). However, gene amplification *via* stepwise increases of the MTX concentration is a time-consuming procedure, and in many cases, it is often difficult to obtain suitable clones because increased specific productivity is prone to be accompanied by metabolic or growth defects [4]. In addition, because the media optimized for one clone may not be effective for other clones when the selected clones are not isogenic (*i.e.*, not derived from the same parental producer cell line), further media optimization, which takes several months using conventional trial-and-error methods, is unavoidable [8]. Furthermore, even when high-producing clones are isolated, their productivity and growth profiles may not be satisfactory in serum-free media. Therefore, selected clones usually need to be adapted to serum-free suspension culture for large-scale production of therapeutic antibodies.

Because antibodies recognizing different epitopes show distinct clinical activities, much effort has been exerted to generate diverse antibodies and screen for higher potency therapeutic antibodies against HGF. Currently, several monoclonal antibodies against HGF have been developed, and their tumor suppressive potency was demonstrated in xenograft mouse models [3, 5, 12, 14]. The recombinant monoclonal antibody SFN68, which we sought to produce in the present work, is unique among HGF-neutralizing antibodies in that it was generated by immunizing the HGF-c-Met complex [11]. The potential advantage of SFN68 over other anti-HGF antibodies elicited by immunizing HGF only appears to be its higher specificity for the HGF-c-Met interaction. The epitope of SFN68 was characterized to be nonlinear, and this novel chimeric monoclonal antibody was shown to block the HGF-c-Met interaction and prevent cell scattering and proliferation [11].

To evaluate the toxicity and anticancer efficacy of SFN68, high-producer cell line establishment is required to provide a substantial amount of antibodies for preclinical and clinical studies. In the present work, we established CHO cell lines producing high levels of the anti-HGF antibody SFN68 using serum-free suspension culture-adapted CHO cells. We report a simple approach for the rapid, effective selection of high anti-HGF antibody-producing clones. Furthermore, we analyzed the growth and antibody production profiles of the selected high-producer CHO cell clones in batch cultures with suboptimized medium.

Materials and Methods

Cell Lines and Cell Culture

The CHO cell lines expressing the anti-HGF monoclonal antibody (mAb) SFN68 were established by transfection of the serum-free suspension culture-adapted DHFR-deficient CHO cell line DG44 (Invitrogen, USA) with an expression vector harboring the cDNA for the anti-HGF mAb SFN68 under the control of the CMV promoter, the DHFR-coding gene required for gene amplification using MTX, and a neomycin resistance gene as a selection marker. The cDNA for SFN68 was designed to produce an intact antibody composed of SFN68 Fab and the human IgG1 Fc fragment [11]. Stable CHO cell lines were selected by growing the transfected cells in the presence of 250 μg/ml G418 for 3–4 weeks. CHO cells were cultured in plates containing CD OptiCHO (Invitrogen) medium supplemented with 8 mM L-glutamine, termed basal medium unless otherwise stated, at 37°C and 5% CO_2. For cultivation in 125 ml Erlenmeyer shaker flasks with agitation at 130 rpm, a Multitron (Infors AG, Switzerland) incubator was used to grow cells under the conditions of 8% CO_2 and 55% humidity. To formulate the suboptimized media, commercially available chemically defined media, including CD OptiCHO (Invitrogen), CDM4CHO (Hyclone, USA), CDM4mab (Hyclone), PowerCHO (Lonza, Belgium), ProCHO5 (Lonza), ExCell 302 (SAFC Biosciences, USA), ExCell DHFR (SAFC Biosciences), and ExCell CD (SAFC Biosciences), were used as basal media. Powerfeed (Lonza), CHO CD Efficient Feed B (Invitrogen), CD Feed 3 (SAFC Biosciences), and Cell Boost kit (Hyclone) were used as feed media. Each feed medium was added to the individual basal media to a final concentration of 15% (v/v) on day 0. All media used for CHO cell cultivation were supplemented with 8 mM L-glutamine.

Selection of SFN68 High Producers by Gene Amplification

The parental SFN68-producing CHO cell line was subjected to multiple passages in CD OptiCHO basal medium containing 500 nM MTX (Sigma-Aldrich, USA). At each passage step, cells were collected by centrifugation, washed, and transferred to fresh medium containing 500 nM MTX. When cells were adapted to the MTX-containing basal medium, they were transferred to 96-well culture plates for isogenic clone isolation by the limiting-dilution method. When subclones formed cell colonies in each well, the anti-HGF mAb concentration in the culture supernatant was measured by enzyme-linked immunosorbent assay (ELISA). The

subclones with higher productivity were propagated in 24-well plates, and their increased productivity was confirmed by measuring their growth rates and antibody titers.

Viable Cell Count and Biochemical Assays

Cell density and viability were determined by trypan blue staining. Glucose, glutamine, and lactate concentrations were measured by enzymatic reactions using a YSI 7100 multi-parameter bioanalytical system (YSI Life Sciences, USA). Parameters representing the metabolic activities of CHO cells were determined by plotting measured values against the integral of viable cells, as described previously [19].

Determination of Anti-HGF Titers

To determine the anti-HGF mAb titer in the culture supernatant, each well of 96-well Maxisorp microtiter plates (Nunc, Denmark) was filled with 100 µl of 1.0 µg/ml goat anti-human IgG Fc (KPL, USA) in 0.1 M carbonate buffer, pH 9.6. After overnight incubation at 4°C, plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and blocked with PBS containing 1% bovine serum albumin (BSA) for 1 h. The plate was washed three times, and 100 µl of culture supernatant was added to each well and incubated for 1 h at 37°C. After washing, plates were incubated with horseradish peroxidase-conjugated goat anti-human IgG antibody (KPL) for 1 h. The plates were washed, after which TMB substrate solution (KPL) was added and color was allowed to develop for 10 min. After stopping the reaction by adding 2 N H_2SO_4, the absorbance was measured at 450 nm using a microplate reader. Data analysis was performed using Gen5 software (BioTek, USA). For quantification by HPLC, purified antibody was bound to the POROS A 20 perfusion-type protein A column (Applied Biosystems, USA) equilibrated with a buffer containing 10 mM sodium phosphate (pH 7.2) and 150 mM NaCl. Bound antibody was step-eluted with 100 mM glycine (pH 2.8) containing 5% acetic acid. Protein was monitored at 280 nm by an ultraviolet detector.

Antibody Purification

After the removal of cells and debris by centrifugation, the clarified culture supernatant was loaded onto a Mabselect SuRe column (GE Healthcare, Sweden) equilibrated with 20 mM sodium phosphate (pH 7.0). After washing with 20 mM sodium phosphate (pH 7.0) containing 1 M NaCl, bound antibody was eluted with 20 mM citrate (pH 3.5). The pH of the eluted fractions was adjusted to 6.5 using 1 M Tris-HCl buffer (pH 9.0).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and ELISA

SDS-PAGE was performed using 4–12% NuPAGE (Invitrogen) Bis-Tris polyacrylamide gels, and proteins were visualized by Coomassie Brilliant Blue staining. For indirect ELISA, microplates (Costar EIA/RIA plate; Corning, USA) were coated with 0.2 µg/ml HGF (R&D Systems, USA). After overnight incubation at 4°C, plates were washed three times with PBS containing 0.05% Tween 20 and blocked with PBS containing 1% BSA for 1 h. The plates were washed three times, and 50 µl of each purified anti-HGF antibody or human IgG (used as a control; Green Cross, Korea) was added to each well by serial dilution. After incubation for 1 h at 37°C, plates were washed and incubated with horseradish peroxidase-conjugated goat anti-human IgG antibody (KPL) for 1 h. After washing, the plates were incubated with ABTS substrate solution (KPL) for 15 min. Absorbance was measured at 405 nm using a microplate reader. The relative HGF-binding activity of the purified antibodies from the selected clones was calculated by a nonlinear method using parallel line analysis in the Gen 5 data processing software (BioTek) to estimate the relative potency (REP), representing the ratio of the binding potency of the antibodies from the selected clones to that of the antibodies from the parental cell line.

Results

Initial Screening for Anti-HGF Antibody SFN68-Producing CHO Cell Lines

The conventional approach for the selection of recombinant protein-producing CHO cells uses adherent cells growing in serum-containing medium, requiring a time-consuming adaptation to serum-free medium after the isolation of gene-amplified high-producer cell lines. In the present work, we attempted to select anti-HGF antibody producers by using the DHFR-deficient DG44 CHO cell line, which was preadapted to serum-free suspension culture, to shorten the clonal selection procedure. As an initial step for the generation of SFN68 producers, the DG44 CHO cells were transfected with an expression vector encoding the chimeric anti-HGF mAb SFN68 to select several hundreds of G418 (neomycin)-resistant cell lines. Evaluation of the anti-HGF production ability of those clones in the CD OptiCHO basal medium led to the isolation of one clone, namely the parental cell line, which produced approximately 1 µg/ml SFN68 in 96-well plates. This parental cell line was further engineered to develop SFN68 high producers by gene amplification in the subsequent experiments.

The G418-resitant parental cell line was subjected to gene amplification in a shaker flask culture containing the basal medium supplemented with 500 nM MTX. In a parallel experiment, the same cell line was also passaged in the absence of MTX. As can be seen in Fig. 1A, more than 3 weeks was required for the parental cell line to become adapted to the MTX-containing medium (>80% cell viability); both cell viability and viable cell counts gradually decreased until the third passage, after which they increased until the sixth passage. Because cells selected with MTX generally

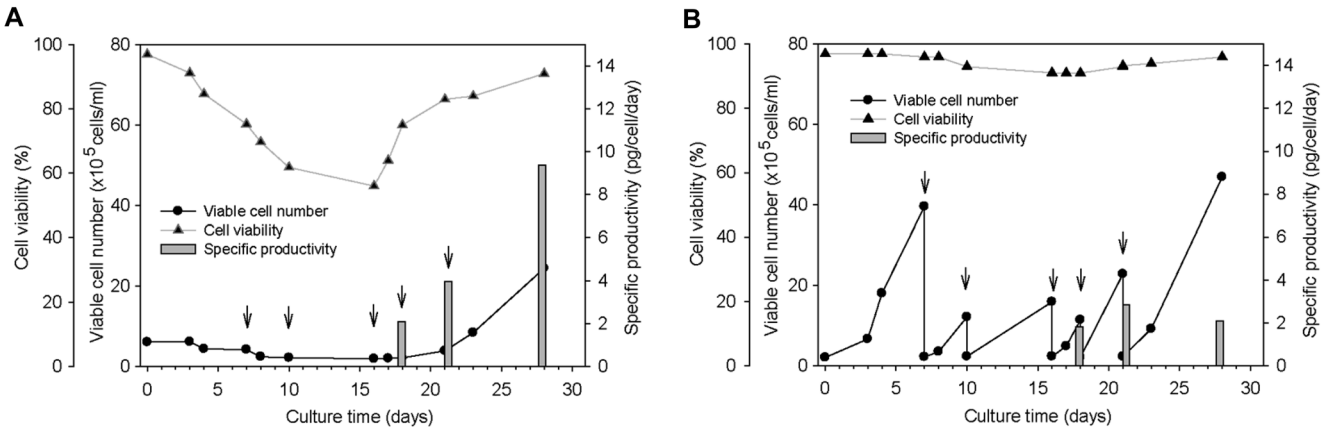

Fig. 1. Growth and antibody production profiles of the anti-HGF mAb SFN68-producing parental CHO cell line during MTX-mediated gene amplification.

(**A**) Cell viability, viable cell number, and the specific productivity were measured during the selection of the gene-amplified clones from the parental SFN68-producing CHO cell line by multiple cell passages in the MTX-containing serum-free CD OptiCHO basal medium. Arrows indicate the days on which cells were passaged for selection of the MTX-resistant clones. A total of five passages were performed under 500 nM MTX-selective pressure. (**B**) A similar experiment was performed in parallel as in (**A**) by cultivating the parental cell line in the absence of MTX.

have a decreased growth rate in spite of their improved productivity [9], we measured the specific productivity during cultivation, rather than volumetric productivity, to ensure that a pool of gene-amplified high producers was enriched. The specific productivity of gene-amplified CHO cell clones at day 28 reached 9.37 pg/cell/day, representing an almost 4.5-fold increase over that of the parental cell line passaged in the absence of MTX (2.08 pg/cell/day) (Fig. 1B).

Evaluation of the SFN68 Production Ability of the CHO Cell Clones Selected by Gene Amplification

Isogenic single-clone isolation was started once the pool of MTX-resistant clones regained a normal growth profile at day 28, by following the experimental scheme shown in Fig. 2A. When cells become adapted to MTX-loaded medium and start to grow rapidly, faster growing cells would become the major population and thereby reduce

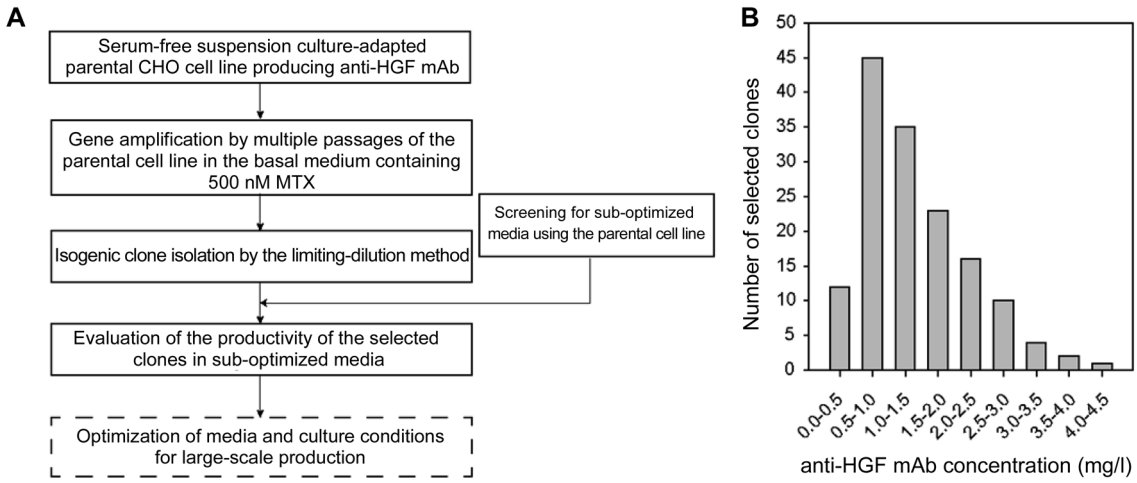

Fig. 2. Efficient selection of CHO cell lines with higher SFN68 productivity.

(**A**) Schematic diagram of the strategy used in the present study for the rapid establishment of anti-HGF mAb-producing cell lines. (**B**) Clonal variation of the gene-amplified SFN68-producing CHO cell lines isolated after MTX-mediated gene amplification. To isolate isogenic CHO cell clones, the MTX-resistant CHO cells obtained from the parental cell line were subjected to clonal isolation by the limiting-dilution method. After growing the diluted cells for 21 days in 96-well plates containing CD OptiCHO basal medium and 500 nM MTX, the culture supernatant was analyzed for the secreted anti-HGF mAb by ELISA.

the chance of isolating the high producers. We therefore immediately harvested the MTX-adapted cells when they started to regain over 90% cell viability at day 28 and subjected them to single-clone isolation by the limiting-dilution method. After 2 or 3 weeks of incubation, colonies resistant to 500 nM MTX formed in 96-well plates containing the basal medium. When the volumetric productivity of each of the selected clones was assessed, we found that most of the selected clones showed an improved productivity ranging from 1.0 to 3.0 μg/ml, with <5% of all clones displaying a higher productivity of 3.0–4.5 μg/ml, even though approximately 40% of the tested clones failed to increase the yield (Fig. 2B).

We assessed the productivity of the two selected clones, 2F7 and 2H4, in the CD OptiCHO basal medium. Those selected clones and the parental cell line were cultivated with shaking in 125 ml Erlenmeyer shaker flasks to compare their growth and production profiles. As shown in Fig. 3, both 2F7 and 2H4 cell lines produced approximately 1.7-fold more anti-HGF mAb at day 14, although the viable cell density decreased by 30% compared with that of the parental cell line, consequently resulting in a 3.2-fold increase in the specific productivity (Table 1). It was also observed that the viability of the 2F7 and 2H4 cell lines was maintained for 2–3 days longer than that of the parental cell line, the viability of which started to drop on day 9. In addition to the gene amplification effect, this prolonged cell viability in part explains the higher productivity of the selected clones in the basal medium.

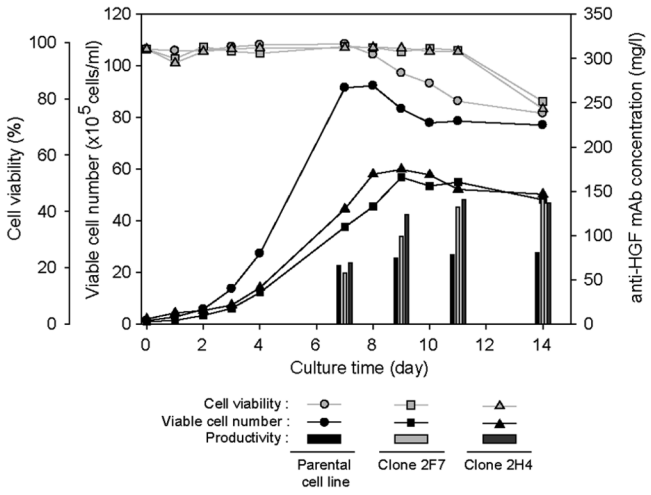

Fig. 3. Cell growth and antibody production profiles of the selected SFN68-producing CHO cell clones in the basal medium.

Two selected clones, 2F7 and 2H4, were cultivated in 125 ml shaker flasks containing CD OptiCHO basal medium for the indicated times. Cell viability, viable cell number, and anti-HGF mAb concentrations were measured as described in the Materials and Methods.

Effect of Feed Medium Supplementation on the SFN68 Production

We tested the effects of supplementation with various feed media on the production of SFN68 using the parental cell lines during the selection of MTX-resistant clones. Diverse media were formulated using commercially available chemically defined media, including the CD OptiCHO basal medium, each of which was combined with diverse feed media to prepare a total of 72 different blended media. As shown in Fig. 4, the parental cell line yielded the highest Ab titer in the medium composed of the CD OptiCHO basal medium and 15% (v/v) of the CHO CD Efficient Feed B after cultivation for 14 days in 125 ml shaker flasks. The maximum anti-HGF antibody titer reached in this suboptimized medium was 490 μg/ml.

Growth and Antibody Production Profiles of the 2F7 and 2H4 Clones in the Suboptimized Medium

We compared the productivity and growth profiles of the selected clones in the suboptimized culture medium we established with the parental cell line. Differing from the growth profiles in the basal medium, the 2F7 and 2H4 cell lines showed growth kinetics similar to that of the parental cell line in the suboptimized medium. In particular, the 2H4 clone, which grew slower than the parental cell line in the basal medium, showed an increased growth rate comparable to that of the parental cell line in the

Table 1. Kinetic parameters of the SFN68-producing parental cell line and the gene-amplified clones in the basal medium.

Cell line	Specific growth rate (day^{-1})	Maximum cell density (cells/ml)	Maximum product concentration (mg/l)	Volumetric productivity (mg/l/day)	Specific productivity (pg/cell/day)
Parental cell line	0.75	7.70E+06	80.33	5.74	1.05
2F7	0.65	5.69E+06	141.37	10.10	3.21
2H4	0.44	5.99E+06	136.38	9.74	3.21

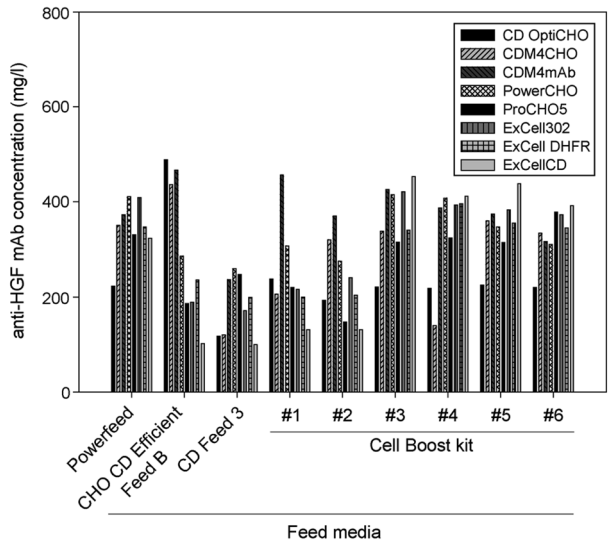

Fig. 4. Effect of feed supplementation with various basal media on the production of SFN68 anti-HGF antibody.
Each of the indicated feed media was added at a final concentration of 15% (v/v) on day 0 to each of the basal media shown in the inset of the figure. The parental SFN68-producing CHO cell line was cultivated for 14 days in 125 ml Erlenmeyer shaker flasks containing the aforementioned media. The anti-HGF mAb titer was determined by ELISA.

suboptimized medium (Table 2). In this medium, the viabilities of the parental cell line and the selected clones were maintained above 80% for up to 14 days (data not shown). As summarized in Table 2, the antibody titers produced by 2F7 and 2H4 cells were 842 and 861 μg/ml, respectively, which were approximately 10-fold higher than that of the parental cell line cultivated in the basal medium (80 μg/ml). The specific productivities of 2F7 and 2H4 cells were 7.78 and 7.33 pg/cell/day, respectively, which represent 1.73- and 1.63-fold increases over that of the parental cell line (4.49 pg/cell/day). The selected clones and parental cell line had similar specific glucose consumption rates. However, there was a noticeable increase in the specific glutamine consumption rate and a >60% decrease in the specific lactate production rate in the 2F7 and 2H4 clones, suggesting that the selected clones indeed display different metabolic properties. Altogether, our simple protocol, in which gene amplification was performed by multiple passages of the parental cells in the MTX-containing media and suboptimized media design was also performed with the parental cell line during high-producer screening, is a feasible time-saving approach that enhances volumetric productivity almost by 10-fold compared with that of the parental cell line cultured in the nonoptimized basal medium (5.74 mg/l/day vs. 52.64 and 53.84 mg/l/day for clones 2F7 and 2H4, respectively).

Analysis of the Binding Properties of the Purified SFN68 Antibody

To investigate the quality of the anti-HGF antibody produced by the 2F7 and 2H4 clones, the antibodies were purified by affinity chromatography using a protein A column. As shown in Fig. 5A, these purified antibodies did not show any differences regarding the molecular weights of the heavy and light chains as well as the complex formed between those two subunits, suggesting that similar degrees of glycosylation might occur in the antibodies. Indirect ELISA revealed that both antibodies produced from the parental cells and those obtained from the 2F7 and 2H4 clones could bind to human HGF with similar affinity (Fig. 5B). The REP, representing the ratio of the binding potency of the antibodies from the selected clones to that of the antibodies from the parental cell line, was determined to be 0.948 and 0.960 for the antibodies secreted by the 2F7 and 2H4 cells, respectively, further suggesting that the antibodies produced by those selected clones are highly similar to that produced by the parental cell line in terms of binding activity.

Discussion

To verify the efficacy of therapeutic antibodies in various cancer models and in clinical trials, their pilot-scale

Table 2. Kinetic parameters of the SFN68-producing parental cell line and the gene-amplified clones in the sub-optimized medium.

Cell line	Specific growth rate (day^{-1})	Maximum cell density (cells/ml)	Maximum product concentration (mg/l)	Volumetric productivity (mg/l/day)	Specific productivity (pg/cell/day)	Specific glucose consumption rate (pg/cell/day)	Specific glutamine consumption rate (pg/cell/day)	Specific lactate production rate (pg/cell/day)
Parental cell line	0.78	1.01E+07	594.10	32.55	4.49	0.64	37.94	143.28
2F7	0.62	1.04E+07	842.27	52.64	7.78	0.54	53.11	54.23
2H4	0.70	1.20E+07	861.46	53.84	7.33	0.50	47.68	47.49

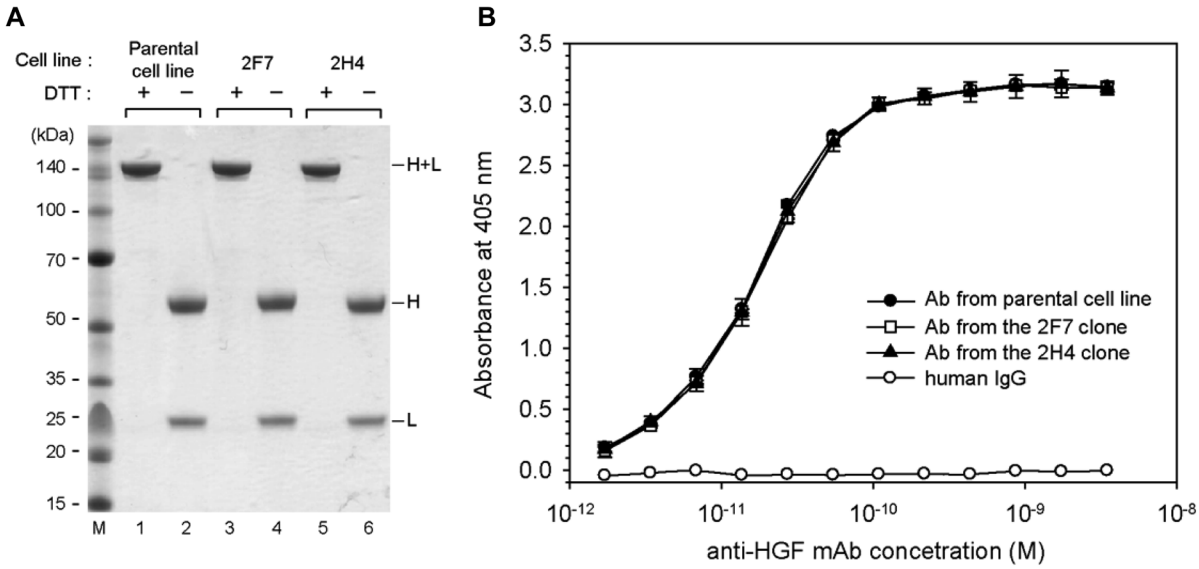

Fig. 5. Analysis of the binding activity of the purified SFN68 produced by the parental cell line and the selected high producers. (**A**) Purified anti-HGF mAb SFN68 from the indicated CHO cell lines was subjected to SDS-PAGE in a reducing (+DTT) or nonreducing (DTT) condition and visualized by Coomassie Blue staining. Protein molecular weight markers (M) are shown on the left in kDa. L, light chain; H, heavy chain. (**B**) Indirect ELISA was performed with increasing concentrations of the purified SFN68 from the high producers 2F7 and 2H4 and human IgG (negative control).

production in a limited time frame is often urgently required. In the present work, we report our successful experimental case in which by starting with CHO cells preadapted to serum-free suspension culture, shortening the selection process for isolating gene-amplified clones, and performing media optimization with the parental cell line in parallel with the high-producer selection process, we were able to rapidly establish anti-HGF mAb SFN68 high producers capable of yielding the substantial amounts of antibodies required for preclinical and early clinical trials.

In the course of the development of biopharmaceuticals, it is very important to shorten the development time, particularly during lead discovery stages when the efficacy and toxicity of candidates need to be evaluated in animal models. The production of therapeutic antibodies requires the establishment of cell lines, gene amplification, subsequent clonal selection followed by adaptation to serum-free suspension culture, and lastly media optimization. This step-by-step process takes several months, generally over 7–8 months, and expression levels are often not satisfactory because the clones selected in serum-containing media and under adherent cell culture conditions are different in terms of metabolic activity in different culture conditions such as serum-free suspension culture. Other approaches such as transient expression or the use of pooled stable cell lines have also been considered for the rapid acquisition of antibodies [1, 15]. Although the transient production of antibodies is an attractive option owing to the short time requirement, the maximum yields are generally much lower than those of stably transfected cell lines. In addition, it is necessary to produce large amounts of plasmid DNA for each transfection. When pooled clones are used, lower production yield and potential protein heterogeneity are longstanding problems. By following our experimental design, in which the parental low-producer cell line was passaged every 2–5 days in medium containing one fixed concentration of MTX (500 nM), we could accelerate the high-producer enrichment process. By this simple gene-amplification method, we could efficiently obtain two SFN68 high producers, 2F7 and 2H4, which showed approximately 3.2-fold higher specific productivity than the parental cell line (1.05 vs. 3.21 pg/cell/day for the selected clone) in the basal medium (Table 1), highlighting the efficiency of this protocol. In general, the process of MTX-mediated gene amplification requires more than five rounds of a serial adaptation process, in which each round of selection step takes 3–4 weeks [17]. MTX, as a folic acid antagonist, blocks the synthesis of purines and pyrimidines. Its half-life in serum was recently determined to be in the range of 6–8 h [23]. Thus, the removal of cellular contents, including purine and pyrimidine biosynthesis intermediates

that would be released from dead cells, by centrifugation followed by washing during cell passages is likely a critical step for the rapid enrichment of high-producer MTX-resistant clones within 4 weeks (Fig. 1A).

To timely produce anti-HGF mAbs using the selected high producers, we prescreened the platform media during the gene amplification procedure. In the present work, commercially available chemically defined media with different ingredients were used because they have advantages over in-house complex media in the production of therapeutic antibodies, when considering regulatory safety issues. Our results showed that the blending of feeds with basal media resulted in varying degrees of increases in the anti-HGF mAb productivity. By supplementing the basal medium with various feed media, we could rapidly select a suboptimized medium yielding a more than 5-fold increase in productivity (Fig. 4). Interestingly, the same feed medium, when combined with different basal media, performed differently, highlighting the importance of testing with multiple basal media during the initial screening for production media. As shown in Table 2, the productivities of the 2F7 and 2H4 cell lines in the suboptimized medium were 842 and 861 mg/l, respectively, which were almost 6-fold higher than those achieved by the selected clones in the basal medium. Although this formulation certainly cannot be the best option for each of the gene-amplified clones, the optimal medium established with the parental cell line was suitable for the selected gene-amplified clones, underscoring that such a simple one-round media screening using commercially available basal and feed media is an effective approach for enhancing antibody production in a short period. Nevertheless, product yields may be further increased by providing minor components limited in the suboptimized medium, removing or reducing the accumulation of toxic by-products, and optimizing other cultivation conditions including agitation and aeration [9, 22]. It is warranted that these factors can be optimized using stirred-jar bioreactors to further increase the productivity of the CHO cell clones selected in the present work.

In summary, we established CHO cell lines producing high titers of the anti-HGF mAb SFN68 by a time-saving gene-amplification process and demonstrated their capability to produce large amounts of SFN68 by applying the selected clones to a suboptimized medium predetermined using the parental cells. The method described in this study can be applied as a simple method for the production of therapeutic antibodies within a limited period to analyze their efficacy in preclinical and clinical studies.

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