METHODS FOR ALTERING PROTEIN PRODUCTION RATES

Field of the Invention

This invention relates to methods for altering the cellular secretion rate of a protein.

Background of the Invention

Large-scale production of proteins, such as antibodies, typically relies on secretion of the protein from a cultured production cell line. Secreted proteins produced by cultured cells can be readily recovered and purified from the surrounding cell culture media.

The cellular secretion rate of proteins is an important parameter affecting the production and purification of secreted proteins from a bioreactor or other system. In general, higher purified protein yields can be attained when the cellular secretion rate is relatively high. Conversely, if the cellular secretion rate is too low protein purification may not be feasible.

One approach to circumventing the problem of low secreting cells has been to isolate high secreting, subcloned cells from a population of low secreting cells. Typically, this requires several time and labor-intensive rounds of limiting serial dilution, screening and selection of high secreting cell lines. Alternatively, entirely new cell lines producing the protein of interest are generated in the hope that the new cell lines will be high secreting lines.

Each of the foregoing approaches to generating high secreting cell lines has limitations. For example, identifying high secreting cell lines by subcloning from a population of low secreting cells is limited by the relative rarity of high secreting cells in the population as well as the extensive amounts of time and labor required for the identification of any high secreting cells.

Further, the generation of new cell lines producing the antibody or protein of interest is limited by the possibility that

the new cell lines will not be high secreting and the substantial amounts of effort required to re-generate antibody producing cells and identify high secreting cells. In some instances, only low secreting cell lines can be obtained due to protein misfolding inside the endoplasmic reticulum (ER) of the cell resulting in a decreased secretion rate.

Thus, a need exists for effective methods of changing the cellular secretion rate of a protein.

Brief Description of the Drawings

Fig. 1 shows UPR gene transcript levels in cells secreting mAbs at high rates.

Fig. 2 shows UPR gene transcript levels in static growth phase cells.

Fig. 3 shows a comparison of UPR gene transcript levels in parent myeloma cell lines.

Fig. 4 shows UPR gene transcript levels in high secreting cell lines relative to the Sp2/0 parent myeloma cell line.

Fig. 5 shows UPR gene transcript levels in high secreting cell lines relative to the FO parent myeloma cell line.

Fig. 6 shows UPR gene transcript levels in high secreting cell lines relative to the Ag-653 parent myeloma cell line.

Fig. 7 shows changes in antibody secretion rates as a function of alteration of UPR gene transcript levels.

Fig. 8 shows increased antibody secretion rates in myeloma cells stably transfected with nucleic acid encoding CHOP-10 specific siRNAs.

Summary of the Invention

One aspect of the invention is a method for altering the cellular secretion rate of a protein comprising the steps of modulating the activity of at least one UPR pathway component in a cell; and culturing the cells.

Another aspect of the invention is a plasma cell with a changed cellular secretion rate generated by altering the cellular secretion rat of a protein comprising the steps of modulating the activity of at least one unfolded protein response (UPR) pathway component in a cell; and culturing the cells.

Another aspect of the invention is a transgenic animal comprising a plasma cell with a changed cellular secretion rate generated by modulating the activity of at least one UPR pathway component in a cell and culturing the cells.

Another aspect of the invention is an isolated nucleic acid having the sequence shown in SEQ ID NO: 15 or SEQ ID NO: 16.

Detailed Description of the Invention

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

The term "antibodies" as used herein is meant in a broad sense and includes immunoglobulin or antibody molecules including polyclonal antibodies, monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies and antibody fragments or variants. Antibodies are secreted proteins constitutively expressed and secreted by plasma cells. Antibodies can also be produced using plasma cells immortalized by standard methods such as hybridoma generation or by transfection of antibody heavy and/or light chain genes into an immortalized B cell such as a myeloma cell or other cell types such as Chinese hamster ovary (CHO) cells, plant cells and insect cells.

Antibody fragments or variants include mimetibodies, Fab fragments, $F(ab')_2$ fragments, Fc fragments, heavy chain fragments,

light chain fragments, and molecules containing a portion of at least one antibody peptide chain. Such portions may correspond to antibody variable, hinge, or constant region peptide chains.

The term "mimetibody" as used herein means a protein having the generic formula (I):

$$(V1(n)-Pep(n)-Flex(n)-V2(n)-pHinge(n)-CH2(n)-CH3(n))(m) \quad (I)$$

where V1 is at least one portion of an N-terminus of an immunoglobulin variable region, Pep is at least one bioactive peptide that binds to an epitope, Flex is polypeptide that provides structural flexibility by allowing the mimetibody to have alternative orientations and binding properties, V2 is at least one portion of a C-terminus of an immunoglobulin variable region, pHinge is at least a portion of an immunoglobulin hinge region, CH2 is at least a portion of an immunoglobulin CH2 constant region and CH3 is at least a portion of an immunoglobulin CH3 constant region, where n and m can be an integer between 1 and 10. A mimetibody can mimic properties and functions of different types of immunoglobulin molecules such as IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgD and IgE dependent on the heavy chain constant domain amino acid sequence present in the construct.

The term "monoclonal antibody" (mAb) as used herein means an antibody (or antibody fragment) obtained from a population of substantially homogeneous antibodies. Monoclonal antibodies are highly specific, typically being directed against a single antigenic determinant. The modifier "monoclonal" indicates the substantially homogeneous character of the antibody and does not require production of the antibody by any particular method. For example, murine mAbs can be made by the hybridoma method of Kohler *et al.*, *Nature 256*: 495 (1975). Chimeric mAbs containing a light chain and heavy chain variable region derived from a donor antibody (typically murine) in association with light and heavy chain constant regions derived from an acceptor antibody (typically another mammalian species such as human) can be prepared by the method disclosed in U.S. Pat. No. 4,816,567. Humanized mAbs having CDRs derived from a

non-human donor immunoglobulin (typically murine) and the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulins, optionally having altered framework support residues to preserve binding affinity, can be obtained by the techniques disclosed in Queen *et al.*, *Proc. Natl Acad Sci (USA), 86*: 10029-10032, (1989) and Hodgson *et al.*, *Bio/Technology, 9*: 421, (1991).

Fully human mAbs lacking any non-human sequences can be prepared from human immunoglobulin transgenic mice by techniques referenced in, *e.g.*, Lonberg *et al.*, *Nature 368*: 856-859, (1994); Fishwild *et al.*, *Nature Biotechnology 14*: 845-851, (1996)' and Mendez *et al.*, *Nature Genetics 15*: 146-156, (1997). Human mAbs can also be prepared and optimized from phage display libraries by techniques referenced in, *e.g.*, Knappik *et al.*, *J. Mol. Biol. 296*: 57-86, (2000) and Krebs *et al.*, *J. Immunol. Meth. 254*: 67-84, (2001).

The term "cellular secretion rate" as used herein means the rate at which a cell secretes a given protein. Such rates may be described as the change in the amount of protein present in the culture media per change in time or can be normalized to cell number and expressed with units "pg/cell/day."

The term "short interfering RNA" as used herein means a nucleic acid sequence that mediates the cleavage of a target gene transcript. Short interfering RNAs (siRNAs) may be double stranded or of the short hairpin type. Double stranded siRNAs may be comprised of two individual, antiparallel, annealed RNA strands or annealed nucleic acid strands which contain both RNA and DNA (*e.g.* 5'-TTTTUUUU-3' annealed to 5'-TTTTUUUU-3' or 5'-TTTT-3' annealed to 5'-UUUU-3'). siRNAs of the short hairpin type may be comprised of a single RNA strand or a single RNA:DNA hybrid strand capable of forming a stem-and-loop structure or other secondary structure effective as an siRNA. Those skilled in the art will recognize that siRNAs may comprise other modifications such as nucleoside analogs, backbone modifications, and other modifications that still permit

the modified siRNA nucleic acid to mediate the cleavage of a target gene transcript.

The term "small molecule" as used herein means a compound with a molecular weight less than 24,000 g/mol which is not comprised solely of amino acid residues or nucleic acid residues.

The term "transcriptional control sequence" as used herein means a nucleic acid sequence which is necessary for the transcription of a gene or a nucleic acid sequence which increases or decreases the transcription of a gene.

The term "UPR pathway component" as used herein and in the claims means peptide chains or nucleic acid sequences, such as transcriptional control sequences, which mediate signaling through the UPR pathway or activate the UPR.

The present invention provides methods useful for altering the cellular secretion rate of a protein by a cell. An exemplary use of the methods of the invention is enhancement of secretion rates for proteins that are useful for therapeutic, diagnostic or research purposes, such as antibodies.

Low protein secretion rates in cell lines can be caused by the accumulation of misfolded proteins in the cell ER slowing or stopping the secretion process via the UPR. Stress-sensing proteins in the ER membrane detect an excess of unfolded protein and trigger the UPR. Subsequently, via a complex signal transduction cascade, a chaperone protein Bip and transcription factors XBP-1 and CHOP are upregulated.

Chaperone proteins bind to unfolded proteins and assist in correct folding. The transcription factor CHOP is generally considered as a negative regulator of cell growth, differentiation and survival. It has been observed that upregulation of CHOP causes cell cycle arrest, thus giving the cell time to cope with the unfavorable conditions responsible for UPR induction.

The transcription factor XBP-1 is required for generating plasma cells, the differentiated B lymphocytes that secrete large amounts of immunoglobulins. Plasma cells display an altered UPR in which an upregulation of some UPR genes occurs prior to

immunoglobulin synthesis. These include XBP-1, Bip, Grp94 and p50ATF6 alpha and their upregulation are necessary for plasma cell differentiation and proper antibody secretion. (Gass, J.N., *et al.*, *J. Biol. Chem. 227*, 49047-49054 (2002). In contrast, CHOP is not upregulated during this transition, suggesting a distinct type of UPR. The upregulation of these molecules can have a beneficial or detrimental effect causing either increases in the cellular protein secretion rate or apoptosis. See Kaufman, R.J., *Genes Dev. 13*, 1211-1233 (1999) and Cudna, R.E., *et al.*, *Biotechnol. Bioeng. 81*, 56-65 (2003).

In a method of the invention, the cellular secretion rate of a protein is altered by modulating the activity of at least one UPR pathway component in a cell and culturing the cell. The method of the invention provides for increasing or decreasing the cellular secretion rate of a protein such as an antibody.

In an embodiment of the invention, the cellular secretion rate of a protein is increased by stably transfecting the cell with a nucleic acid encoding a UPR pathway component. UPR pathway components may be polypeptides or nucleic acid sequences, such as a transcriptional control sequence, which mediate signaling through the UPR pathway or activate the UPR. Examples of UPR pathway components include BiP, XBP and CHOP and variants having similar activity. Other examples of UPR pathway components include IRE1, PERK, ATF4, ATF6, eIF2alpha, GRP78, GRP94, calreticulin, chaperones, and variants having similar activity (*see e.g.* Cudna and Dickson, *Biotechnol. Bioeng.*, *81*, 56-65 (2002)). An example of a transcriptional control sequence is the cis-acting UPR element (UPRE) and ERSE which have been identified in the promoters of different UPR genes. Those of ordinary skill in the art will recognize other UPR pathway components and transcriptional control sequences. The UPR pathway component can have an amino acid sequence as shown in SEQ ID NO: 2 (murine BiP), SEQ ID NO: 4 (murine XBP-1, spliced form), SEQ ID NO: 6 (murine XBP-1, unspliced form), SEQ ID NO: 8 (murine CHOP-10), SEQ ID NO: 10 (human BiP), SEQ ID NO: 12 (human XBP-1) or SEQ ID NO: 14 (human CHOP-10). The UPR pathway

component nucleic acid can have a sequence as shown in SEQ ID NO: 1 (murine BiP mRNA), SEQ ID NO: 3 (murine XBP-1, spliced form mRNA), SEQ ID NO: 5 (murine XBP-1, unspliced form, mRNA), SEQ ID NO: 7 (murine CHOP-10 mRNA), SEQ ID NO: 9 (human BiP mRNA), SEQ ID NO: 11 (human XBP-1 mRNA), SEQ ID NO: 13 (human CHOP-10 mRNA).

Variants of these sequences having an activity similar to the parent molecule will also be useful in the methods of the invention. For example, variant molecules having at least 80% identity to the parent molecule would be expected to have similar activity. Percent identity between two protein sequences can be determined using the BLASTP algorithm with filtering turned off and all other default settings unchanged. Different isoforms of a polypeptide, dominant negative versions of a polypeptide, or covalently modified forms of a polypeptide are some examples of variants of a parent molecule.

In another embodiment of the invention, the cellular secretion rate of a protein can be decreased by decreasing the expression of a UPR pathway component. Gene expression of a UPR pathway component such as BiP or CHOP can be decreased by of a cell with short interfering RNA (siRNA) molecules or antisense molecules.

In another embodiment of the invention, the cellular secretion rate of a protein is increased by modulating the UPR pathway component through administration of a small molecule. An exemplary small molecule is thapsigargin, a UPR induction agent (Litton, J., *J. Biol. Chem. 26*, 17067-17071 (1991). Other examples of such small molecules include tunicamycin and lipopolysaccharide.

In another embodiment of the invention, the cellular secretion rate of a protein is increased by placing the cells in static growth phase. Cells may be placed in static growth phase by limiting nutrient availability, allowing cellular wastes to accumulate or changing the pH of the cell culture media. Those skilled in the art will also recognize other methods for placing cells in the static growth phase.

In the methods of the invention, exemplary cells are plasma cells, *i.e.*, differentiated B-cells that secrete antibodies. Plasma cells may be isolated from murine, human, or other animal sources.

Typically, the plasma cells have been immortalized by standard techniques such as viral infection, with Epstein-Barr Virus, *e.g.*, or other methods such as radiological or chemical mutagenesis. The immortalized plasma cells can also be cancerous and can be obtained by injecting mineral oil or another compound, into the peritoneal cavity of an animal.

In one embodiment of the invention, the immortalized fusion partners are what is known in the art as "myeloma cells." Myelomas are generally formed from the fusion of spleen cells with an immortalized fusion partner obtained from an organism suffering from multiple myeloma, a bone marrow cancer. The organism can be birds, fishes, reptiles, mammals and other *Animalia*. Examples of myeloma cell lines include the SP2/0 (American Type Culture Collection (ATCC), Manasas, VA, CRL-1581), NSO (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646), and Ag653 (ATCC CRL-1580) cell lines which were obtained from mice. An example of a myeloma cell line obtained from humans is the U266 cell line (ATTC CRL-TIB-196). Those skilled in the art will recognize other myeloma cell lines.

In one embodiment of the invention the myeloma cells are stably transfected with a DNA molecule. Stably transfected myeloma cells may be generated by methods of transfection, screening and selection well known to those of ordinary skill in the art. DNA sequences used to stably transfect the cells may be randomly integrated into the DNA of a myeloma cell or integrated in a site-specific manner. Such DNA sequences may encode UPR pathway components. Additionally, the stably transfected nucleic acid sequences may insertionally inactivate or delete a UPR component such as a UPR gene or transcriptional control sequence.

In another embodiment of the invention, the cellular secretion rate of a protein is increased by stably transfecting the cell with a nucleic acid sequence encoding a siRNA that targets transcripts of a nucleic acid sequence encoding a UPR pathway component. Such siRNAs may target transcripts of a nucleic acid sequence encoding a CHOP protein. Exemplary CHOP proteins are those having the amino

acid sequences shown in SEQ ID NO: 8 or SEQ ID NO: 14. Stable transfection with a nucleic acid sequence encoding a siRNA which targets transcripts of nucleic acid sequences encoding murine CHOP-10 are useful in the methods of the invention. Exemplary murine CHOP-10 gene transcript specific siRNA comprise the nucleotide sequences shown in SEQ ID NO: 15 or SEQ ID NO: 16. Those skilled in the art will recognize other nucleic acids encoding siRNAs that target UPR gene transcripts.

Other cells useful in the methods of the invention include Chinese Hamster Ovary (CHO) cells, insect cells and plant cells.

In another embodiment of the invention, transgenic animals constitutively or inducibly expressing UPR proteins at elevated levels relevant to non-transgenic animals can be produced. Techniques for producing transgenic animals are known in the art.

In the methods of the invention, the cells are cultured. Cells may be cultured in suspension or as adherent cultures. Cells may be cultured in a variety of vessels including, for example, bioreactors, cell bags, culture plates, flasks and other vessels well known to those of ordinary skill in the art. Cells may be cultured in any suitable media including chemically defined media formulations. Ambient conditions suitable for cell culture, such as temperature and atmospheric composition, are also well known to those skilled in the art. Methods for the culture of cells are also well known to those skilled in the art.

The present invention also provides plasma cells with changed cellular secretion rates generated by the methods of the invention. The plasma cells may be generated by modulating the activity of a UPR pathway component with a stably transfected nucleic acid encoding a short interfering RNA. The siRNA may target transcripts of a nucleic acid sequence encoding a UPR pathway component such as a CHOP protein. The plasma cell provided may be, for example, a SP2/0 derived cell such as a C2-8 or C2-18 cell. Such plasma cells may secrete an antibody or other polypeptide to be purified.

The present invention will now be described with reference to the following specific, non-limiting Examples.

Example 1

UPR Gene Transcript Levels in High Secreting Cell Lines

Cell lines that secrete mAbs at high rates were analyzed for levels of UPR gene transcripts compared to cell lines secreting an identical mAb at low rates. The cell lines examined included the high secreting lines C505B, C505C and C505D. The low secreting lines examined were the C505A line and SP2/0, the parent myeloma cell line of C505A, B, C and D. All of these lines were transfected with DNA encoding the heavy and light chains of a human IgG1 mAb specific for αv-integrin. The C505B, C505C and C505D cell lines were identified by sequential subcloning, screening and selection for high rates of mAb secretion.

Antibody secretion rates were determined by measuring the amount of mAb secreted in a 24 hour period into the cell culture medium and counting the number of viable cells to generate a cell secretion rate with units of "pg/viable cells/day." The C505a cell line secretes antibody at low rates of 5-7 pg/viable cell/day, equivalent to a concentration of about 5-10 μg/mL/7 days. The C505B line produces antibody at a rate of about 15 pg/viable cell/day, C505C produces at a rate of about 13 pg/viable cell/day, and C505D produces at a rate of about 15 pg/viable cell/day.

All mAb secreting cell lines and the parent myeloma cell line were cultured in suspension at 37°C in IMDM media containing 5% FBS, 2 mM glutamine, and 2 mM pyruvate in an atmosphere of 5% CO_2. 1X MHX selection medium containing 0.5 mg/L mycophenolic acid, 2.5 mg/L hypoxanthine and 50 mg/L xanthine was also present in the media.

Differences in UPR gene transcript levels between the high and low expressing cell lines were assessed via quantitative PCR (Q-PCR). Cells were grown into the exponential growth phase and total RNA was isolated from $5x10^6$ cells using the RNEasy™ system (Qiagen Inc., Valencia, CA). Q-PCR was performed using a standard two-step reaction. The cDNA synthesis step was pérformed using Superscript™

II reverse transcriptase (Invitrogen Inc., Carlsbad, CA) and random hexamer primers using reaction conditions specified by the manufacturer. Taqman™ Q-PCR (Applied Biosystems, Foster City, CA) was then performed with ABI PRISM™ 7000HT or 7900HT instrumentation (Applied Biosystems, Foster City, CA) as specified by the manufacturer. 5,000 pg of RNA was used in each Q-PCR reaction. BiP, CHOP, and XBP specific primers and probe combinations used for Q-PCR were designed using Primer Express™ software (Applied Biosystems, Foster City, CA). cDNA levels were normalized against transcript levels for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene and then normalized to the cDNA/GAPDH transcript value for the appropriate parent cell line. Data collection and transcript quantitation in the early exponential phase of the PCR was performed using the ABI PRISM 7000HT or 7900HT instrumentation and associated software.

The results are shown in Fig. 1 and show that BiP and XBP-1 UPR gene transcript levels were about 3-4 times greater in high secreting cell lines than in low secreting cells. Differences in CHOP levels were less pronounced.

Example 2

UPR Gene Transcript Levels and Antibody Secretion Rates in Static Growth Phase Cells

Cells in the static growth phase were analyzed for levels of UPR gene transcripts and mAb secretion rates relative to cells in the exponential growth phase. The C168J cell line is a transfectoma derived from the SP2/0 parent myeloma cell line and secretes IgG1 mAb at a rate of 25-30 pg/cell/day. C505A is as described above in Example 1.

The IgG1 mAb secretion rates of the C505A, C168J, and SP2/0 cell lines were assessed by seeding $5x10^6$ cells in T-75 or T-150 flasks and culturing as described in Example 1. After 3 days cells were in the exponential growth phase and at 6 days cells were in the static growth phase. Total cell number and viable cell number were determined for cells in both growth phases. Culture media from cells in both growth

phases was assayed for human IgG by standard enzyme linked immunosorbent assay (ELISA). BiP, CHOP, and XBP-1 specific Q-PCR was performed for exponential and static growth phase cells as described in Example 1 above.

The results shown in Table 1 demonstrate that the C505A and C168j lines have increased IgG1 mAb secretion rates (Table 1).

Table 1: Rate of Secreted IgG1 (pg/cell/day)

Cell line	Exponential Phase	Static Phase
C505A	3.90	5.40
C168j	10.40	13.90
SP2/0	0	0

Further, the results shown in Fig. 2 demonstrate increased UPR gene transcript levels in C505A, C168j and Sp2/0 in static phase growth relative to exponential phase growth.

Example 3

UPR Gene Transcript Levels in Parent Myeloma Cell Lines

UPR gene transcript levels in the SP2/0, NSO, FO, and Ag653 parental myeloma cell lines were examined to determine whether higher secreting cell lines contain higher levels of UPR genes. SP2/0, NSO, FO, and Ag653 cells were grown into the exponential growth phase and total RNA was isolated from $5x10^6$ cells as described in Example 1 above. Q-PCR and analysis was also performed as described in Example 1. The results shown in Fig. 3 show comparable BiP and CHOP transcript levels in all cells examined. However, XBP-1 transcript levels were elevated in FO and NSO cells relative to the other cell types examined.

Example 4

UPR Gene Transcript Levels in High Secreting Cell Lines and Parent Myeloma Cell Lines

Cell lines that secrete mAbs at high rates were analyzed for levels of UPR gene transcripts compared to their parental cell lines. The cell lines indicated in Figs. 4-6 were grown into the exponential growth phase and total RNA was isolated from $5x10^6$ cells as described in Example 1 above. Q-PCR and analysis was also performed as described in Example 1.

The results shown in Figs. 4-6 shows that cell liness secreting mAbs at higher rates have higher UPR gene transcript levels relative to their SP2/0, FO, and Ag653 parent myeloma cell lines. These results suggest that increased UPR gene expression is coupled with increased antibody secretion rates regardless of the identity of the parent myeloma cell line used to generate the high secreting cells.

Example 5

UPR Protein Expression After UPR Induction

UPR protein expression in high secreting cells relative to low secreting cells and parent myeloma cell lines were analyzed after UPR induction by the pharmacological agent thapsigargin. Thapsigargin is an ATPase inhibitor that blocks sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps and leads to calcium depletion in the ER lumen.

Cells were treated with 100 nM of thapsigargin and XBP-1 and CHOP-specific Western blots prepared. Cells were lysed at defined timepoints in radioimmunoprecipitation (RIPA) lysis buffer (1X PBS, 1% N-P40, 0.5% Sodium deoxycholate, 0.1% Sodium dodecylsulfate, 1 mM PMSF, and protease inhibitors from Roche (Catalog No. 1836153) and protein concentrations in the clarified lysates were quantitated using a standard bicinchoninic acid (BCA) protein assay (Sigma Catalog. No. B9643). For Western blots, 20 µg of each lysate was run on a standard SDS-PAGE gel, transferred to polyvinylidene fluoride

(PVDF) membranes, and probed with antibodies specific for XBP-1 or CHOP (Santa Cruz Biotechnology Inc., Santa Cruz, CA).

The results (not shown) indicated that UPR proteins in high secreting cells are expressed at high levels relative to low secreting cells and parent myeloma cell lines. The XBP-1-specific Western blot indicates that XBP-1 protein expression is strongly induced in thapsigargin treated, high secreting C168j cells. This induction of XBP-1 expression is most pronounced 7 hours after pharmacological induction of the UPR, and is visible as early as 2 hours post-treatment. The CHOP-specific Western blot indicates that CHOP protein expression is strongly induced in thapsigargin treated, high secreting C168j cells. CHOP first appears at 2 hours, is maximally expressed at 4 to 7 hours and is much reduced by 22 hours.

Example 6

Increasing UPR Protein Expression Levels

Cells were transiently transfected with BiP and XBP-1 expression vectors in an attempt to elevate cellular expression levels of these UPR proteins. HEK293T/17 (ATCC CRL-11268) cells were grown as adherent cultures at 37°C in an atmosphere of 5% CO_2 and cultured in Iscove's Modified Dubelcco's Minimal Essential Media (IMDM) media containing 5% FBS, 2 mM glutamine, and 2 mM pyruvate. cDNAs encoding XBP-1 isoform 1 (NCBI Accession AF027963), XBP-1 isoform 2 (NCBI Accession AF443192), or BiP were subcloned using standard methods into a vector useful for transient transfection experiments. The vector expresses the zsGreen1 protein under the control of a CMV promoter and has a multiple cloning site for introduction of an additional coding region under the control of a CMV promoter. A kanamycin resistance gene was used for bacterial selection. HEK293T/17 cells were then either left untransfected or transiently transfected with the empty vector alone, XBP-1 isoform 1 expression vector, XBP-1 isoform 2 expression vectors or BiP expression vector. Transfections were performed using the Lipofectamine™ 2000 reagent (Invitrogen Inc., Carlsbad, CA) as directed by the manufacturer. Forty-eight hours following

transfection the cells were lysed in RIPA lysis buffer and equal cell equivalents of clarified lysates were loaded onto SDS-PAGE gels. Western blots were then prepared and probed as described in Example 5 above. Blots were then stripped and reprobed with an actin-specific antibody to confirm equal protein loading in each lane.

The results (not shown) demonstrated that XBP-1 isoform 1 (lane labeled "XBP-1") and XBP-1 isoform 2 (lane labeled "X54") expression levels were elevated in cells transiently transfected with expression vectors encoding these XBP-1 isoforms. Further, the data (not shown) indicated that BiP expression levels were elevated in cells transiently transfected with expression vectors encoding Bip and in the cells left untransfected or transfected with empty vector alone, no BiP expression was detected.

Example 7

Effect of UPR Gene Transcript Levels on Antibody Secretion Rates

The effect of UPR gene transcript levels on antibody secretion rates was analyzed. Double-stranded siRNA molecules targeted to BiP, CHOP, and XBP-1 gene transcripts were designed using Ambion's internet based siRNA Target Finder Tool (www.ambion.com/techlib/misc/siRNA_finder.html) and were synthesized using the Silencer™ siRNA Construction Kit (Ambion Inc., Woodward, TX, Catalog No. 11620). Two siRNAs were designed for each targeted transcript. $3x10^6$ C168J cells cultured under standard conditions were transfected via electroporation with 3 μg of each of these double-stranded siRNAs as indicated in Fig. 10. Electroporated cells were suspended in 2 ml of IMDM media as described in Example 1 above and then cultured on T-12 plates. Viable cell concentrations were determined 8 days later. IgG1 mAb concentrations were also determined 8 days later using standard nephelometry techniques.

The results in Fig. 7 show that antibody secretion rates can be modulated by altering UPR gene transcript levels on antibody secretion rates. Transfection of high secreting C168J cells with short interfering RNAs (siRNA) capable of preventing expression of

the UPR genes BiP, CHOP, and XBP-1 isoforms from RNA transcripts decreased C168J cell IgG1 secretion rates.

Example 8

Increasing Antibody Secretion Rates by Increasing UPR Gene Transcript and Expression Levels

Over-expression of UPR proteins can increase the secretion rates of proteins such as mAbs by cells. Protein secreting cell lines, such as mAb secreting cell lines, are transfected with expression vector constructs encoding BiP, CHOP, XBP-1, and other UPR associated proteins to effect the over-expression of these proteins. Cells are transfected with these expression vector constructs either individually or in combination. Appropriate protein secretion rates, such as antibody secretion rates, are determined at 2, 4 and 6 days after transfection using standard techniques. Protein secretion rates in transfected cells are compared to the secretion rates of non-transfected control cells. Protein secretion rates are expected to be higher in cells over-expressing one or more UPR proteins. In the event that constitutive overexpression of Bip or Xbp-1 eventually induces apoptosis in these cells, these genes can be placed downstream of an inducible promoter and activated only when needed.

Example 9

Increasing Antibody Secretion Rates in Myeloma Cells Stably Transfected with CHOP-10 Encoding siRNA

Stable transfection of C465A myeloma cells with DNA constructs producing CHOP-10 specific siRNAs increased antibody secretion rates (Fig. 8). Two different siRNA molecules targeted to CHOP-10 gene transcripts were designed using Ambion's internet based siRNA Target Finder Tool (www.ambion.com/techlib/misc/siRNA_finder.html). The siRNA molecules designed were of the short hairpin type. Nucleic acids encoding these siRNAs (SEQ ID NO: 15 and SEQ ID NO: 16) were synthesized and cloned into the BamHI and HindIII sites of the pSILENCER4.1-neo vector (Ambion Inc., Woodward, TX) using standard methods. Cloning of the nucleotide sequence shown in SEQ ID NO: 15

into pSILENCER4.1-neo produced pCHOP1, while cloning of the sequences shown in SEQ ID NO: 16 into this vector produced pCHOP2. The pCHOP1, pCHOP2, and pSILENCER4.1-neo plasmid DNAs were then each separately transfected into C465A myeloma cells by electroporation. Stably transfected myeloma cells containing these vectors were then selected in SFM8 media containing 10% FBS and 300 μg/ml G418 by culture at 37°C in an atmosphere of 5% CO_2. This selection media also contained 1X MHX selection medium containing 0.5 mg/L mycophenolic acid, 2.5 mg/L hypoxanthine and 50 mg/L xanthine to maintain stable antibody expression by the C465A cells.

Each cell line indicated in Fig. 8 was grown in suspension for six days in SFM8 media containing 10% FBS, 1X MHX and 300 μg/ml G418. Viable cell density and antibody titers were determined daily during this six day period. Viable cell density was assayed by standard dye exclusion assays and antibody titer in the culture media was assayed by standard nephelometry techniques. The C465A myeloma cell line is derived from the SP2/0 myeloma cell line and stably expresses a human TNF-alpha specific murine mAb of the IgG1 kappa isotype. The C2-8 myeloma cell line is a C465A derived cell line stably transfected with pCHOP1. The C2-18 myeloma cell line is a C465A derived cell line stably transfected with pCHOP2. The V11 and V12 cell lines are C465A derived cell lines stably transfected with pSILENCER4.1-neo alone; both lines were independently generated.

The data obtained indicated that the CHOP-10 specific siRNAs encoded by pCHOP1 and pCHOP2 increased antibody specific productivity relative to the control C465A, V11, and V12 cell lines (Fig. 8).

The present invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit and scope of the appended claims.

CLAIMS

1. A method for altering the cellular secretion rate of a protein comprising the steps of:

a) modulating the activity of at least one unfolded protein response (UPR) pathway component in a cell; and

b) culturing the cells.

2. The method of claim 1 wherein the cellular secretion rate is increased.

3. The method of claim 2 wherein the UPR pathway component is modulated by stably transfecting the cell with a nucleic acid encoding a UPR pathway component.

4. The method of claim 3 wherein the UPR pathway component nucleic acid encodes a protein having the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13.

5. The method of claim 4 wherein the UPR pathway component nucleic acid has a sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.

6. The method of claim 3 wherein the UPR pathway component is a transcriptional control sequence.

7. The method of claim 2 wherein the UPR pathway component is modulated by administering a small molecule.

8. The method of claim 7 wherein the small molecule is thapsigargin, tunicamycin or lipopolysaccharide.

9. The method of claim 3 wherein the UPR pathway component is BiP, XBP- or CHOP.

10. The method of claim 2 wherein the UPR pathway component is modulated by placing the cells in static growth phase.

11. The method of claim 1 wherein the cell is a myeloma cell.

12. The method of claim 11 wherein the myeloma cell is Sp2/0, NS0, F0 or Ag653.

13. The method of claim 11 wherein the myeloma cell is a subclone of Sp2/0, NS0, F0 or Ag653.

14. The method of claim 1 wherein the protein is an antibody.

15. The method of claim 1 wherein the cellular secretion rate is decreased.

16. The method of claim 15 wherein the UPR pathway component is modulated by stably transfecting the cell with a nucleic acid encoding a short interfering RNA (siRNA) that targets transcripts of a nucleic acid encoding a UPR pathway component.

17. The method of claim 2 wherein the UPR pathway component is modulated by stably transfecting the cell with a nucleic acid encoding a siRNA that targets transcripts of a nucleic acid sequence encoding a UPR pathway component.

18. The method of claim 17 wherein the siRNA targets transcripts of a nucleic acid sequence encoding a CHOP protein.

19. The method of claim 18 wherein the CHOP protein has the amino acid sequence shown in SEQ ID NO: 8 or SEQ ID NO: 14.

20. The method of claim 18 wherein the nucleic acid comprises the nucleic acid sequence shown in SEQ ID NO: 15 or SEQ ID NO: 16.

21. A plasma cell with an altered cellular secretion rate generated by the steps of:

a) modulating the activity of at least one UPR pathway component in a cell; and

b) culturing the cells.

22. The plasma cell of claim 21 generated by modulating the activity of a UPR pathway component by stably transfecting the cell with a nucleic acid encoding a siRNA that targets transcripts of a nucleic acid sequenc encoding a UPR pathway component.

23. The plasma cell of claim 22 which is a SP2/0 derived cell comprisin a stably transfected nucleic acid encoding a siRNA that targets transcripts of a nucleic acid sequence encoding a CHOP protein.

24. The plasma cell of claim 23 that secretes an antibody.

25. The plasma cell of claim 23 that is a C2-8 or C2-18 cell.

26. A transgenic animal comprising the plasma cell of claim 21.

27. An isolated nucleic acid sequence comprising the nucleic acid sequence shown in SEQ ID NO: 15.

28. An isolated nucleic acid sequence comprising the nucleic acid sequence shown in SEQ ID NO: 16.