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Promoters of Cancer Genes for Recombinant Protein Expression in Human Cancer Cell Lines

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ABSTRACT

Introduction: Production of complex human recombinant proteins is an important issue in medical biotechnology. These proteins are mostly expressed in non-human mammalian host cells. This has some problems including non-human post-translational modifications, application of high-cost agents for inducing protein expression and low yields. Thus, it is necessary to use new expression systems to overcome the indicated challenges. ***Methods:*** In this paper, we hypothesize the application of promoter regions of cancer genes, which have a high rate of transcription in human cancer cell lines, for designing new expression vectors. ***Results:*** After designing, these vectors could be applied to produce complex human recombinant proteins in the human cancer cell lines as production hosts. ***Conclusion:*** Application of these expression vectors for the production of recombinant human proteins in the human cancer cell lines have some advantages including authentic post-translational modifications, proper-cost of commercialization, and high yields.

Introduction

Mammalian cells have currently been considered important hosts for the production of recombinant human complex proteins. Production of recombinant proteins in these cells comprises more than 50% of human protein market (Wurm 2004); and other host cells, especially prokaryotic cells, are unable to produce functional human recombinant proteins because of incorrect folding and incorrect post-translational modifications on desired proteins (Demain *et al* 2009). In addition, about 70% of recombinant therapeutic proteins are produced in the mammalian cells and this portion is increasing. Also, some of the commercially important proteins can only be produced in the mammalian cell lines (Reichert *et al* 2005).

Despite the mentioned advantages, the mammalian hosts themselves have also some disadvantages for the production of human recombinant proteins. For example, mammalian expression vectors use viral-based promotes for the expression of cloned genes. Most of these expression vectors produce relatively low amounts of protein with higher costs of large-scale bio-processing compared to the prokaryotic expression vectors (Demain *et al* 2009). To increase the production rate of recombinant protein in the mammalian hosts, many inducible vectors have been used (Fieck *et al* 1992). These vectors are usually used for the production of cytotoxic and cytostatic recombinant proteins. These types of vectors were constructed based-on heat shock control (Schweinfest *et al* 1988), metal ion control (Hu *et al* 1990), steroid control (Ko *et al* 1989) and with some successes bacterial transcriptional control systems induced by IPTG (Fieck *et al* 1992). Unfortunately, the induction levels were low (Yarranton *et al* 1992). However, inducible vectors designed based on modified lac operator produced higher levels of protein in response to IPTG induction (Labow *et al* 1990, Baim *et al* 1991).

Besides some advantages of inducible mammalian expression vectors, application of high-cost compounds and time-consuming processes for their induction are disadvantages of such inducible vectors. Therefore, it is important to design vectors that are induced at high rates by low-cost substances.

Non-human mammalian cell lines, especially CHO cells, are usually used for the commercial production of human therapeutic proteins (Walsh 2006). Despite the advan-

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tages of these cell lines over other hosts, there was a critical problem with using of non-human mammalian host cells. This problem is the improper glycosylation of produced proteins in these hosts. This incorrect glycosylation which could induce immune responses in the patients, causes their rapid clearance from the bloodstream, and affects correct folding, solubility and biological activity of the protein products (Sethuraman and Stadheim 2006, Jenkins *et al* 1996). For example, because of the inability of CHO cells in adding 2-6-sialyl-galactose, therapeutic proteins produced in these cells are rapidly cleared from the bloodstream (Jenkins *et al* 1996).

Hypothesis

The hosts

The main drawbacks associated with CHO cells for recombinant protein production, can be avoided by using human-originated hosts. These hypothesized hosts have some intrinsic advantages; one advantage is their ability in performing 100% correct post-translational modifications on the product recombinant proteins. Other advantage is the adhesion of some of the human host cancer cells to the surface of cell culture containers. This property allows the easy collection of host cells' supernatant for the purification of produced proteins. Therefore, it minimizes the impurity of recombinant protein with host proteins during the purification processes. As another advantage, the resistance of human cancer cell lines to the removal of serum from their culture medium enables their cultivation in the serum-free media and by using serum-free media, the concentration of non-product proteins reaches to the minimum levels in the supernatant of cultured host cells. These two main advantages lead to the higher purity of isolated recombinant proteins by simplifying the purification process. Also, the adhesion of host cells to the surface of container minimizes the loss of seeded host cells in the bioreactor for starting new bioreaction therefore, removing the need for reseeding of bioreactors. The aforementioned advantages (i.e. simplified purification of recombinant products, possibility of serum-free cultivation and minimum loss of seeded hosts) reduce the cost of recombinant protein production in the human cancer cell lines. Transient gene expression is one of the important strategies used to produce large-yields of recombinant proteins in a short timeline from mammalian cells as hosts (Baldi *et al*, 2007). Because of existence of many similarities among human cancer cell lines and other mammalian host cell lines, transient gene expression could be used for large-scale production of human recombinant proteins in the human cancer cells in the future experimental works and is another main factor for reducing the cost of commercial protein production using human cancer cell lines. Some studies have reported the use of immortalized human cells for the production of recombinant proteins (Dong *et al* 2006, Pau *et al* 2001). These reports support our hypothesis on using human cancer cell lines as novel hosts for the production of human complex recombinant proteins.

Because of potential risks associated with recombinant products which are produced from tumor cell lines, the produced proteins may be used in *ex vivo* or *in vitro* applications such as using in cell culture supplementary culture media. If the recombinant proteins are therapeutic proteins, the immortalized human cells should be used as hosts instead of human tumor cell lines for protein production. This is because the production of human recombinant proteins in the cancer cell lines may do not be compatible with regulations of Food and Drug Administration (FDA) (see *important considerations* section for more explanations).

The vectors

Some genes, known as cancer genes, have the high expression rates in the human cancer cell lines compared to normal cells. Some of these genes include human telomerase reverse transcriptase, tyrosinase, survivin and epidermal growth factor receptor. The expression rates of hTERT in cancer cell lines are about 70-100 folds higher than those of normal cells, and sometimes its promoter activity is equivalent to that of the SV40 promoters (Takakura *et al* 1999). Another study showed that in lung cancer cell lines, the activity of hTERT promoter is varied from %70 to %120 of CMV promoter activity (Uchino *et al* 2005). Similarly, the rates of tyrosinase expression are 10-10000 folds higher in melanoma cell lines than those in normal cells. In addition, the levels of tyrosinase promoter activity are about 100-1000 times more compared to those of SV40 promoter activity (Ulasov *et al* 2007). The expression of survivin, another cancer gene, is about 100-1000 times higher in the cancer cell lines than in the normal cells (Chen *et al* 2004). Results of a research study showed that activity of survivin promoter is nearly equivalent to that of the SV40 promoter in the cancer cell lines (Yang *et al* 2004). In a study conducted by Vallian *et al* (1998), it has been shown that the activity of EGFR promoter is about two-folds higher than that of CMV promoter. The EGFR gene is constantly over-expressed in the cancer cell lines compared with normal cells (Voldborg *et al* 1997). The high expression rates of aforementioned cancer genes are because of high activity of their promoters. Therefore, the promoters of cancer genes can be used as promoters of customized expression vectors. These vectors can be applied in the production of recombinant human proteins in the human cancer cell line as host (discussed in the vector section of hypothesis) in which the used promoter has the highest activity.

Most of the cancer gene promoters have response elements for various enhancers (Nishi *et al* 2004, Rubins-

tein *et al* 1998, Bertolotto *et al* 1996, Ling *et al* 2004). This property can be exploited to increase the expression of interested genes. Although the activity of cancer gene promoters is increased by binding of some intracellular proteins to their related response elements located on these promoters, but it may influence promoters of other genes in the host cell line. Therefore, this issue should be considered in the designing of new expression vectors. As an example, the activity of promoter of human telomerase reverse transcriptase (hTERT) gene is increased in the hypoxia conditions through binding of HIF1 (hypoxia inducible factor-1) to the hypoxia response elements (Nishi *et al* 2004). The increasing of hTERT promoter activity by hypoxia may be advantageous in the bioreactors where enough oxygenation is an important challenge (Garcia-Ochoa and Gomez 2009). If the activity of used promoters is increased in response to the hypoxia, the limited oxygenation would not be problematic, and even could be a favorable factor for the cloned transgene transcription. In addition, increasing of hTERT promoter activity by hypoxia removes the need for using of the high-cost substances in the large-scale processes and can have the commercial advantages.

Activity of commercial promoters, especially viral ones, can be decreased, or ablated, during the next generations of host cell lines. This is caused by some mechanisms like the positional effects related to the epigenetics of host cell lines (Prösch *et al* 1996). This phenomenon is very rare about the promoters of cancer genes because cancer genes play central roles in the life of cancer cell lines and therefore, inactivation of their promoters is impossible by epigenetic mechanisms (they have long-term high expression levels in the cancer cells in comparison with other genes). This property is considered an important advantage for cancer genes promoters against viral ones. Table 1 shows some candidate cancer genes which their promoter regions can be utilized for constructing novel expression vectors. The main features of our proposed expression systems for the production of human recombinant proteins have been compared with those of common protein expression systems in Table 2 (Barzegari *et al* 2010).

Table 1. Examples of promoters used for designing novel expression vectors

Promoter	Host cell line(s)	Inducer	Reference
Tyrosinase	Melonoma cell lines	cAMP*elevating agents	Bertolotto *et al* 1996
EGFR**	Many cancer cell lines	Tricostatin A	Rubinstein *et al* 1998
hTERT***	Most cancer cell lines	Hypoxia	Nishi *et al* 2004
Survivin	Most cancer cell lines	Hypoxia	Yang *et al* 2004

*Cyclic Adenosine Mono Phosphate
**Epidermal Growth Factor Receptor
***Human Telomerase Reverse Transcriptase

Table 2 (A). Comparison of the hypothesized protein expression system with common expression systems: Main characteristics of commercial protein expression systems compared with our proposed system

System	Commercialization cost	Human specific post-translational modifications	Final yield	Risks	Time of scaling up
Bacteria	Low	No	High	Toxin	Short
Yeast	Medium	Similar	High	Low	Short
Insect	High	Similar	Medium	Low	Medium
Non-human Mammalian cells	High	Similar	Medium	Virus	Medium/short***
Plant cell	Low	Similar	Similar	Low	Medium
Human cancer cell lines	Low*/medium	Identical	Medium/ high**	Virus, oncogenes	Medium/short***

*The cost of commercialization can be low or medium depending on selected cancer gene promoter, host cell line and type of inducers for production of human recombinant proteins.
**The Final yield can be medium or high depending on selected cancer gene promoter, host cell line, type of inducers and density of the cultivated host cell line for production of human recombinant proteins.
***The time needed for scaling up can be significantly reduced by using transient gene expression method (Baldi *et al* 2007).

Table 2 (B). Comparison of the hypothesized protein expression system with common expression systems: Comparison of main features of viral and cancer genes promoters

Promoter	Transcription rate	Reguability by low-cost procedures\agents	Inactivation in the next generations of host cell lines
Viral	Medium/high	Low	Probable*
Cancer genes	Medium/high**	High	Approximately impossible*

*Inactivation of viral promoters may be because of epigenetic phenomena of host cell lines (see the text of paper for more details).
**Transcription rate of promoters of cancer genes can be improved by addition of extra inducer-responsive elements as well as recombination of them with viral promoters.

Important considerations

Four important points should be considered in the designing of hypothesized protein expression systems. These points include:

Instability of transfected vectors in the next generations of host cell lines

The vectors should be integrated into the genome of host cell lines because plasmid vectors cannot be transferred to the next generations of host cell lines and therefore, are not stable for a long time inside the host cells. This instability may lead to the interruption in the process of recombinant protein production and thus limitation of the final yield of the recombinant products. For addressing this challenge, the cloned gene must be integrated to the genome of host cell line. This guarantees the stability of transgene and high-yield production of interested proteins.

Lower expression rate of transgene from cancer gene promoters compared with the commercial promoters like viral ones

In some cases, promoters of cancer genes may have lower rates of activity compared with commercial ones particularly viral promoters. This problem can be overcome by increasing of inducibility capacity of the promoters of cancer genes, leading in increased expression rates from such promoters. Increasing of inducibility capacity can be achieved by addition of inducer-responsive sequences and removing of silencer elements from the nucleotide sequence of used cancer gene promoters. As an example, the addition of hypoxia-responsive elements to the sequence of hTERT promoter boosts transcription rates of cloned genes by induction of hypoxia in the cultured host cell lines. Beside addition of extra inducer-responsive sequences and removing of the silencers from the sequence of cancer gene promoters, viral promoters can also be recombined with promoters of cancer genes to construct the more powerful promoters. In addition, the mentioned methods can be applied simultaneously for the construction of even more powerful promoters.

Lower yields of recombinant human proteins from human cancer cell lines in comparison with other hosts due to slower growth of human cancer cell lines

One of the major reasons for lower yields of recombinant human proteins from human cancer cell lines may be related to their longer doubling times compared with those of commercial hosts. Longer doubling times may make human cancer cell lines low-yield hosts for the production of human recombinant proteins. This problem is considered an important limitation for our hypothesis and it may be resolved through engineering of host cell lines by using advanced molecular biology techniques. Other method is the use of richer growth media. Engineered host cell lines may be also cultivated in richer media to increase the final yields of recombinant protein expression even more than using rich media or engineered host cell lines alone. Furthermore, large-scale bioprocesses can be designed so that host cancer cell lines' density reaches to the maximum possible levels, allowing for accumulated amounts of recombinant proteins. Maximizing the amount of recombinant proteins by this method can compensate for longer doubling times of host cancer cell lines. As another way, one can insert more than one copy of the interested gene into the vector. This strategy could also increase the amount of translated protein.

Health risks related to the impurities

The purified protein products may be contaminated with oncogenes, viruses, viral DNA, etc. from host cell lines. These impurities may cause diseases in consumers. This problem limits *in vivo* applications of recombinant products. Therefore, impurities must be removed by implementation of strict quality control processes (Good Manufacturing practice or GMP). In addition, produced proteins can be used for *in vitro* applications like using in supplementary media for cultivation of stem cells, development of ELISA techniques for detection of them in the biological samples, etc. In addition, it is possible to use the indicated vectors in the production of recombinant proteins from immortalized human cells as hosts and this strategy is compatible with FDA regulations.

Conclusion

Considering the useful features of promoter regions of cancer genes for the construction of new expression vectors as well as potentials of human cancer cell lines for producing authentic human recombinant proteins, new expression systems can be designed using mentioned constructed expression vectors and human cancer cell lines, as the host for producing complex human recombinant proteins. These proposed expression systems need to be improved by the integration of cloned gene into the genome of host cancer cell lines, modifications of promoters to the higher rates of transcription and engineering of host cell lines to faster growth. The proposed expression systems could be tested by the conduction of experimental studies in the future.

Ethical issues

Not applicable for this paper.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

MPM and BF developed the idea of this hypothesis and prepared the draft of manuscript. KNK and TM gathered related literature and organized them as the first draft. MPM and TM studied, edited and verified final version of the manuscript.

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