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Cancer cells: novel expression systems in pharmaceutical biotechnology

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Abstract

Every day, numerous medications are used worldwide to treat different kinds of diseases. A huge part of drug manufacturing - is done in pharmaceutical biotechnology companies. Scientists have developed a variety of methods to synthesize these substances. They can insert the gene or the cDNA of a desired protein into special expression systems and extract the resulted products using different methods. The paraneoplastic syndromes are signs and symptoms originated from cancer cell derived products and not because of direct invasion of tumor cells or metastasis. Cancer cells can secret a wide variety of products such as growth hormones, antibodies and so on. In an innovative route, these products may be processed further and eventually be used as useful biologic substances. In this manuscript, we described a process by which scientists can use cancer cells in order to produce various types of biological substances which can be used as medications, diagnostic substances and research materials. Our hypothesis has been inspired from autonomous production of biologic substances from those cancer cells that are responsible for manifestations of paraneoplastic syndromes.

Keywords
Paraneoplastic syndromes, Cancer cell, Biotechnology, Expression system

Introduction

Pharmaceutical products are the backbone of medicinal therapy. Drugs, vaccines, immunoglobulins, hormones, blood products and lots of therapeutic and diagnostic substances are made in biotechnology plants. There are various methods for manufacturing these substances. Genetic engineering using different recombinant expression systems is frequently used in the companies. Scientists insert a gene or cDNA coding for a protein of interest into a well defined expression system (1). There are various types of expression systems including bacteria (2), fungi and yeast (3), plant systems (4) and transgenic animals (5). These systems start transcribing mRNA from the new gene or cDNA which is followed by translation of mRNA to protein. Next, the desired protein should be recov-

ered, purified and finally formulated into the final product. Based on the expression system that is used, various techniques and laboratory methods are needed for each of these phases.

Harmful effects of both benign and malignant cancer cells are not only due to their mass effects or metastasis, but also because of production of some hormones, growth factors and cytokines which can cause different signs and symptoms known as paraneoplastic syndromes (6, 7). Some of the clinical manifestations of such hormones and cytokines are hypercalcemia (8), Cushing syndrome (9) and gynecomastia (10) due to the overproduction of parathyroid hormone-related peptide (PTHrP), adrenocorticotropic hormone (ACTH) and beta human chorionic gonadotropin (beta-hCG) respectively. In some cases, the production of antibodies from cancer cells cross reacting with normal cellular components are the origin of the complex symptoms of tumors (11) including neurologic and ophthalmologic manifestations.

In this manuscript, we introduce those cancer cells which are responsible for paraneoplastic syndromes, as new expression systems suitable for use in pharmaceutical biotechnology.

The ***Hypotheses***

For manufacturing biologic substances, scientists insert the gene or cDNA of a special protein into some well characterized expression systems, extract the product of these systems and process it further to finally use it as a medication or diagnostic tool. Some cancers cells produce biologic substances such as hormones, cytokines, antibodies and so on which can be used as drugs such as growth hormones. At this point, we review some products of cancer cells that are believed to be responsible for manifestations of paraneoplastic syndrome.

Paraneoplastic polycytemia is originated from erythropoietin production from renal cell carcinoma (12). In addition, some cancers including small cell (13) and non small cell (14) lung cancers secrete anti diuretic hormone (ADH) which can lead to the syndrome of inappropriate ADH secretion. It is reported that ACTH was released from squamous cell carcinoma of prostate (15) and pheochromocytoma (16). Furthermore, it has been elucidated that corticotrophin-releasing hormone (CRH) from Wilms' tumor of kidney could cause Cushing syndrome via stimulating the production of ACTH (9). Patients affected with high grade leiomyosarcoma of the spermatic cord may show manifestations of gynecomastia due to secretion of beta-hCG (10). Escobar et al., 2007 reported that gastrointestinal stromal tumor could secret insulin-like growth factor II, via a mutation in exon 9 of the tyrosine kinase receptor which caused severe hypoglycemia (17). Granulocyte colony stimulating factor (G-CSF) is a glycoprotein which could stimulate the proliferation and maturation of hematopoietic cells in the bone marrow. A number of cancers such as cholangiocarcinoma (18) could secrete G-CSF that leads to leukemoid reaction. Fibroblast growth factor has been accused for oncogenic osteomalacia which is a kind of paraneoplastic syndrome. Some cancers including phosphaturic mesenchymal tumor of mixed connective tissue could result in this syndrome (19). Dirnhofer et al., 2000 assessed the trophoblastic hormone production of different types of lung cancers and showed that alpha subunit of human chorionic gonadotropin (HCG) were secreted from these tumors (20).

One of the mechanisms of oncogenic hypercalcemia is the production of PTHrP from cancer cells. Squamous cell carcinoma of hypopharynx (8) could produce PTHrP. A complete list of tumors responsible for endocrine paraneoplastic syndromes via production of hormones are reviewed by DeLellis and Xia , 2003 (7).

In addition to produce factors causing paraneoplastic endocrine syndromes, tumor cells are rich repertoires of antibodies. A list of these antibodies is summarized by Alabduljalil and Behbahani, 2007 (21). To sum up, we hypothesize that those cancer cells which are responsible for features of paraneoplastic syndromes could be used for manufacturing various types of biologic substances.

Evaluation of the Hypotheses

For evaluating the hypotheses, we designed a study which is depicted in figure 1. In collaboration with clinicians, patients suffering from cancers who are suspected to have a paraneoplastic syndrome should be screened for possible pathogenic products such as hormones, cytokines, antibodies etc. After finding the responsible substance, the surgical specimen, which is routinely excised from these patients, should be send to laboratory for further processes. The specimen should be minced into smaller pieces and be enzymatically digested with collagenase. The separated cells should be conveyed to a new culture medium. After 24 hours, the cells should be distributed between several separated plates as a small population of cancer cells may produce the expected products. After 24 hours, the condition media of the cells which now contain the substances secreted from the cultured cells should be extracted and be assessed for the level of the product of interest. Each plate with the highest level of the product should be selected and its cells should be conveyed to a bioreactor for mass culturing of the desired cells as well as mass production of the product. For quantity evaluation, the researcher may statistically compare the concentration of newly synthesized protein between cancer cell cultures and normal cell cultures, which are engineered to produce the desired product, using student's t-test. The product of the bioreactor should be processed further and be analyzed for quality control and the results could be compared to other expression

systems by student's t-test or Mann Whitney u-test for non parametric variables. Finally, the desired product should be formulated and packaged for commercial purposes.

Discussion

Here, we briefly review some benefits of cancer cell culture. First of all, cancer cell culture is easier than normal cell culture. Cancer cells divide more rapidly than normal cells (22). Normal cells have only a limited number of divisions before they die because of having telomeres. The telomeres get shorter and shorter as a cell divides, and seem to play a role in programming when a cell should die. Some cancer cells live infinitely by synthesizing telomerase enzyme that keeps the telomeres from shrinking and extends them (23). Normal cells will stop growing when they start to touch other cells, a mechanism which is called contact inhibition; while cancer cells do not stop growing when they reach each other which makes it possible to increase their number (24). Normal human cells are hard to grow in the laboratory and require a complex nutritional base with some growth factors; while cancer cells have simpler need for nutrition, reduced need for growth factors and get lots of their requirements for growth factors via an autocrine route. Tumor cells are able to grow at concentrations of growth factors which are insufficient for normal cell proliferation (25). Another advantage of paraneoplastic responsible cancer cells over other current expression systems is that we can simplify the manufacturing process through bypassing the steps of inserting the responsible gene or cDNA into cancer cells because they are producing the desirable products themselves. In other words, they are automated expression systems which make the products autonomously. Lots of the products of these tumor cells are used in clinical medicine as medications, diagnostic markers or research materials. Erythropoietin is used to treat anemia in patients who are under dialysis (26). ACTH is a good treatment for infantile spasms (27). CRH is used as a diagnostic test for assessment of hypothalamic-pituitary-axis (28). G-CSF is frequently used in stem cell transplantation (29). Other biologic substances have usages in diagnostic or research areas. From a commercial and practical viewpoint, someone may ask how the quality of these cancer derived products is. Researchers in the field of paraneoplastic syndromes have used routine biochemistry kits to detect the products of these cancer cells (10) which shows a similarity between cancer derived biological substances and those that are currently used as drugs or diagnostic materials. Nonetheless, some kinds of cancer cells produce special types of biologic substances. For example, Escobar et al., 2007 reported a patient with severe paraneoplastic hypoglycemia. The tumor analysis showed excessive production of insulin-like growth factor II m-RNA and the precursor protein, "big" insulin-like growth factor II (17). Although some kinds of cancer cells produce different types of one special substance, these materials still work; because they result in some effects which can lead to clinical manifestations of paraneoplastic syndromes such as Cushing's syndrome and polycytemia. Besides, different types of a special substance which have higher potency to cause clinical manifestations could be used as novel alternatives of drugs and increase the number of different variants of one drug.

In conclusion, we suggest a new approach to drug manufacturing which is easy and cost beneficial and offers excellent opportunity to employ cancer cells in service and favor of medical and biological sciences.

Conflict of interest

The authors declare they have no conflict of interest.

Overview Box

First Question: What do we already know about the subject?

The paraneoplastic syndromes are signs and symptoms originated from cancer cell derived biologic substances such as hormones and cytokines. Pharmaceutical biotechnology uses different methods to manufacture useful biologic substances such as drugs using various expression systems.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?

We suggested that cancer cells responsible for paraneoplastic syndromes could be used as automated expression systems; i.e. they could produce biologic substances without need for inserting external genes into them. This new method of drug manufacturing seems to be cost effective and easier.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?

To test the hypothesis, cancer cells of surgical biopsy of patients with a defined paraneoplastic syndrome should be cultured in laboratory and the cells with the most secretion capability should be determined and conveyed to bioreactors for mass production of that substance. Quality, quantity and safety of product should be determined and cost benefit analysis of procedure should be performed.

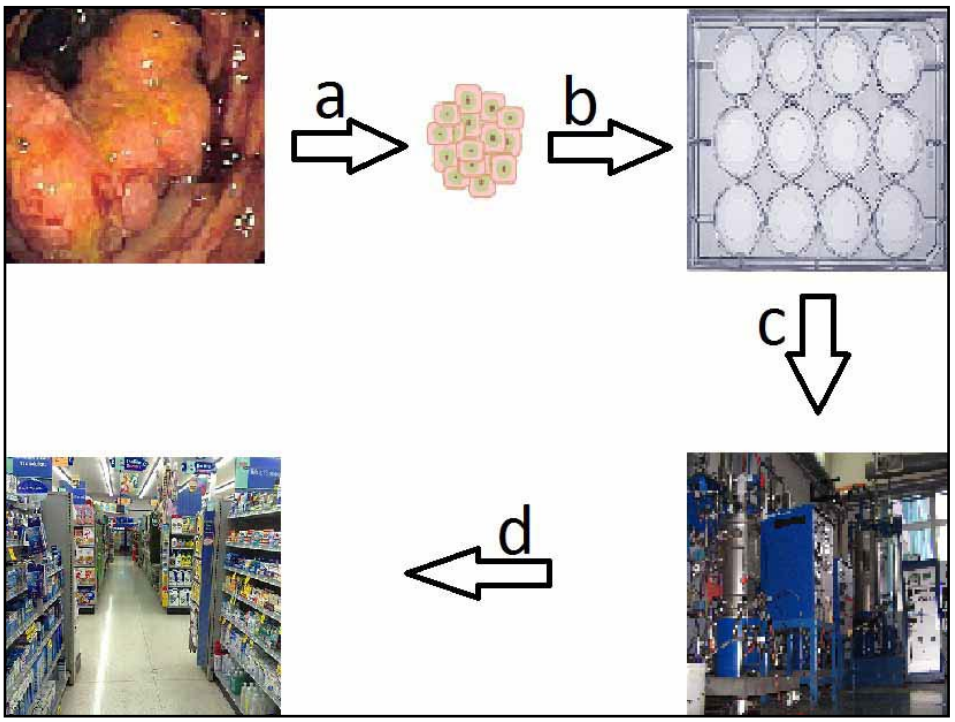

Figure 1. Schematic representation of the steps of our hypothetical procedure. a) After surgical resection of tumor or getting a biopsy, the specimen should be sent to the laboratory, minced to small pieces and digested by collagenase to separate the cells. b) After 24 hours, cells should be conveyed to a 12 well culture plate and after residing in the incubator for 24 hours, the level of desired substance should be determined in each well. c) Each plate with the highest level of the product should be selected and its cells should be conveyed to a bioreactor for mass production of the substance. d) The product of the bioreactor should be processed further and after quality and safety approval, it could be used in drugstores, hospitals and diagnostic or research laboratories.

In courtesy of (clockwise starting from top left):
http://www.topnews.in/health/files/cancer_4.jpg;
http://en.wikipedia.org/wiki/File:Cancer_requires_multiple_mutations_from_NIHen.png;
http://www.glass-bottom-dishes.com/images/12-well.JPG;
http://www.fkkksa.utm.my/bio/images/lab10.jpg;
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