Application of Proteomics to the Study of Hepatocellular Carcinoma and Some Related Diseases

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ABSTRACT Hepatocellular carcinoma is a malignant tumor causing one of the highest death rates in the world. Viral hepatitis, hepatic fibrosis and hepatocirrhosis etc. are some of the most important causes of hepatocellular carcinoma. With the advent of the post-genomic age, studying carcinoma and some related diseases using the developing technology of proteomics has become a major focus of researchers. This article is a review of the application of proteomics to study hepatocellular carcinoma and some related diseases.

KEYWORDS: *proteomics, hepatocellular carcinoma, hepatic fibrosis, hepatocirrhosis.*

With the establishment of the human genomic framework and advent of the post-genomic age, life science research has turned to proteomics from genomics. The term "proteomics" was coined by Wilkins and Williams as early as 1994 to describe the large-scale characterization of all the proteins of a cell or tissues.[1] Therefore proteomics is the study of the proteome, which not only includes the proteins translated by mRNA directly, but also the proteins which are processed post-transcriptionally.[2] Thus the level of mRNA is not equivalent to the cellular proteins. Recent analysis of human liver indicates that the correlation coefficient between the amount of mRNA and abundance of corresponding proteins is only 0.48.[3] To really understand human diseases, we should study the proteins which function in life activity.

Today, applying the technology of proteomics to study tumors has become a major focus of research. In Africa, southeast Asia and China,the incidence of hepatocellular carcinoma has ranked fifth in incidence of tumors. In China, the number of people suffering from hepatocellular carcinoma has increased by 110,000 annually with males accounting for 80,700 and females 29,500. The incidence has risen to 23.7/10,000. A review of the application of proteomics to study hepatocellular carcinoma and some related diseases follows.

Technology of Proteomic Research

Two-dimensional electrophoresis (2-DE), mass spectrometry (MS) and bioinformatics are the most important technologies of proteomics.

Two-dimensional electrophoresis

2-DE sprang up in the 1970s. The first separation of proteins is based on their isoelectric point while the second is based on molecular

Received September 20, 2005; accepted October 17, 2005.

weight in SDS-PAGE. The separated proteins are thus displayed as spots instead of strips. Earlier, procedures used ampholytes for the first separation. This has many disadvantages including complexity in operation, excessive time in focusing etc. Researchers have improved reproducibility and resolution of the procedure by replacing the ampholytes with an immobilized pH gradient(IPG), thus achieving a better sepatation of the proteins. The proteins separated by 2-DE are scanned into a computer and analysed by PDQuest or Image Master. Through these procedures one can detect different proteins between normal and pathologic states.

Mass spectrometry

MS has developed in recent years to such an extent that it can analyze and identify different proteins. The technique involves ionization of the samples, separation of the proteins and determination of their relative molecular weights. MS can be conducted by two different methods: matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) and electrospray mass spectrometry.

Bioinformatics

The main task of bioinformatics combined with proteomics is to separate and identfy the different proteins. The core of bioinformatics is the establishment and perfection of protein databases. At present many institutions in different countries and regions are developing their own protein databases for convenience of finding different proteins that various cells or tissues in different states express, such as the SWISS-PROT and 2-DE protein database of the liver cancer cell line (http://proteome.btc.nus. edu.sg/hccm) that Liang et al. created.[4]

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Hepatocellular carcinoma

The formation of hepatocellular carcinoma involves many factors. At present the core of research includes the mechanism of formation of hepatocellular carcinoma, identifying the new tumor markers and screening for more effective drug targets.

Inquiry into the mechanism of formation of hepatocellular carcinoma

It is well known that the development of hepatocellular carcinoma occurs over a long period of time, progressing in stages that are influenced by many factors. One may say that it is in essence a type of genetic disease, which results from activated oncogenes and inactivation anti-oncogenes. But the variety of these genes will translate into various proteins. Since proteomics focuses on all proteins in the cell, it provides a powerful tool to study the mechanism of hepatocellular carcinoma formation.

Yokoyama et al.[5] made use of 2-DE and time of flight mass spectrometry to analyze hepatocellular carcinomas and adjacent tisssue. They identified 11 different proteins.Through MALID-TOF and a database of peptide mass fingerprinting they identified the absence of 8 proteins in hepatocellular carcinoma such as liver type aldolase, tropomyosin beta-chain, ketohexokinase, enoyl-CoA hydratase, ferritin light chain and arginase 1 etc. These proteins mostly are related to abnormal function of liver cells and the development of hepatocellular carcinoma.

Seow et al.[6] using 2-DE, coupled with silver dye staining and analysis with MALDI-TOF-MS, indentified about 400 proteins in liver cancer cell lines, among them mostly housekeeping proteins such as alcohol dehydrogenase and glucose-6-phosphate dehydrogenase. In addition, they identified some proteins involved with development of hepatocellular carcinoma including the 14-3-3 proteins, annexin and thioredoxin peroxidase. The recognition of unique proteins in tumors will certainly contribute to the understanding of the mechanism of hepatocellular carcinoma formation.

Identifying new tumor markers for hepatocellular carcinoma

The factors related to the formation of hepatocellular carcinoma currently include B and C hepatitis viral infection, hepatocirrhosis, chemical toxins and the absence of chemical nutrients etc. A cell in the process of changing from a normal state into a pathologic one undergoes various changes in protein expression, and so by using the technology of proteomics one can display the differences in a protein expression map based on the development of a tumor from a normal state of the tissue.

Poon and Johnson[7] conducted research on serological markers for hepatocellular carcinoma in patients, and discovered, in addition to a high level of AFP, the traditional tumor marker, high levels of C4 and ceruloplasmin. Of interest was that they found a unique form of AFP associated with primary hepatocellular carcinoma (PHCC). They compared the maps of protein ex-

pression before and after operation, and discovered that the isomer of the particular AFP in the map of protein expression disappeared after the operation. So Poon and Johnson suggested that the particular isomer of AFP probably was a tumor marker.

Steel et al.[8] collected the sera from hepatocellular carcinoma patients, those with active and inactive HBV infection but without clinical symptoms, and conducted 2-DE to identify proteins by peptide mass fingerprinting. They found that the expression levels of 2 types of proteins in the sera of hepatocellular carcinoma patients were higher, namely C3-c fragment and the isomer of apoA1. These researches all demonstrated that we can apply the technology of proteomics to look for and identify some new tumor markers to diagnose hepatocellular carcinoma and other liver diseases.

Looking for drug targets to cure hepatocellular carcinoma

Looking for effective therapeatic agents and drug targets is one of the most extensive applications of proteomics. By comparing different protein expression in abnormal states with the normal one aims to identify effective drug targets. Ding et al.[9] proposed the reason for invasion and metastasis of hepatocellular carcinoma was that it secreted some special proteins. To study this proposal they applied proteomic technology to the liver cancer cell lines MHCC97H, with a high metastastic rate, and MHCC97L with low a metastastic rate. Their results showed that cytokeratin19 (CK19) was expressed at a high level in MHCC97H cells but at a low level in MHCC97L cells. Using radioimmunoassay to study mouse hepatocellular carcinoma,they further found that the level of CK19 increased along with the progression of the tumor, and CK19 showed a marked increment when lung metastasis from the hepatocellular carcinoma occurred. They further used immunohistochemistry to confirm that in 102 patients with obvious liver metastasis from hepatocellular carcinoma, CK19 was positive in their sera. So a protein of this type may become a drug target,with the goal of producing some protein inhibitors that can be employed clinically as drug targets to defer or arrest metastasis.

Related hepatocellular carcinoma diseases

Viral hepatitis

Viral hepatitis has main types, A B C D E. Among them, the hepatitis B virus is the major cause of viral hepatitis. A chronic infection of the virus will induce hepatic pathology, eg., hepatic fibrosis, hepatocirrhosis and PHCC. Based on statistics, about 10%~25% of people with a chronic hepatitis B virus infection will eventually develop HCC.[10,11] To search serum for markers with a high specificity and sensitivity for the hepatitis B virus, He et al.[12] compared sera from patients infected with HBV with normal people by using proteomics technology. They identified some proteins which showed an obvious change in patients' sera such as haptoglobin, haptoglobin beta and alpha 2 chain, apolipoprotein A-I, IV, α_1-antitrypsin and transthyretin. These proteins all showed changes in expression quantity and expression type.They suggested that combined measurement of the quantity and type of these proteins would contribute to diagnosing and treating HBV.

Hepatic fibrosis and hepatocirrhosis

Hepatic fibrosis and hepatocirrhosis contribute to widespread liver damage. They are caused by various diseases that are chronic or recurring and play an important role in development of hepatocellular carcinoma. Domestic and international experts have held a consistent opinion on reversibility of hepatic fibrosis, and they have gradually approved of the reversibility of hepatocirrhosis. But assessment of hepatic fibrosis and hepatocirrhosis depends on a liver biopsy, and so a method of diagnosis without causing trauma has been a major point of concern. Xu et al.[13] applied proteomics technology to analyzing hepatic fibrosis and hepatocirrhosis. Using a normal rat model they detected a specific protein that maintained expression and found that it shared homology with a histidine-rich glycoprotein. By comparing 2-DE results of tissues from normal liver, hepatocellular carcinoma, hepatic fibrosis and hepatocirrhosis, Lim et al.[14] also found changes in 21 meaningful proteins. Among them sarcosine dehydrogenase, liver carboxylesterase, peptidylprolyl isomerase and lamin B1 showed distinct alterations in expression in HCC. But lamin B1 showed a gradual rising in hepatocirrhosis and hepatocellular carcinoma tissues and in hepatocirrhosis the level of expression was the hightest, so it hoped that it can be used as a serum marker to diagnose hepatic fibrosis. These researches cited above portend of a breakthrough in diagnosing hepatic fibrosis and hepatocirrhosis, thus suggesting a means to prevent developing into hepatocellular carcinoma. Currently researchers indicate that hepatic fibrosis and hepatocirrhosis are caused by an accumulation of extracellular matrix, especially collagen. Since liver stellate cells are the main

cells that produce the liver extracellular matrix, including collagen, research on these cells is of great interest.[15] Kristensen et al.[16] found that 27 kinds of proteins have the same expression in a static or actived state by setting up protein maps of liver stellate cells of rats. For example there was a high expression level of calcyclin and low level of expression of serine protease inhibitor.These studies expand further the relationship among hepatic fibrosis, hepatocirrhosis with the liver stellate cells at the protein level.

Problem and Outlook

After proteomics became one of the main research tools of the post-gene age, it has been applied to the study of various diseases. Research on liver diseases using proteomics has concentrated on difference analysis based on 2-DE and MS. 2-DE separates and authenticates proteins of low abundance, extreme acidity and alkalinity and difficult solubility. However these proteins expressed at a low level play important roles in life processes. Because of inadequate reproducibility and sensitivity of 2-DE, there may be variable results from different laboratories with the same sample. But the technique of separation of samples recently has made marked progress, for example, procedures to get rid of high-abundant proteins, such as using narrow IPG gel strips and protein chips. Currently 16 countries and regions have already formulated "The Mankind Liver Proteomics Plan" led by Fuchu He Academician in China. Its goal is to display and identify proteins of the liver. Through the setting up of proteomic maps of normal and pathologic liver, we can provide important scienfic foundation for disease prevention, diagnosis, therapy and development of new therapeutic agents. Liver diseases have a high incidence rate in China. Therefore we should not lose this opportunity to make use of proteomics technology to carry on related basic and applied research on liver diseases.

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