CHAPTER-1

INTRODUCTION
Hepatocellular carcinoma (HCC) arises from the hepatocytes, the major cell type in liver. According to the World Health Organization, HCC is one of the leading causes of death worldwide accounting for 13% of all cancer related deaths. Liver cancers accounted for 662,000 deaths in 2008 and were the third leading cause of cancer-related deaths, exceeded only by cancers of the lungs and stomach (WHO report, 2008). It is the fifth most common cancer in the world with a 5 year survival rate of less than 5% and an incidence of at least one million new patients per year (El-Serag et al., 2007; Christopher et al., 2009; Chantragan et al., 2010). The incidence in the western countries is rising, mainly because of alcoholic liver disease and hepatitis C infection. The cumulative life time risk of liver is 0.88% in men and 0.42% in women; and increase in HCC has been driven by an increasing proportion of those patients developing cirrhosis over the time (Chantragan et al., 2010).

Major risk factors for liver cancer include hepatitis viral infection (B and C), food additives, alcohol, aflatoxins, environmental and industrial toxic chemicals, air and water pollutants (Farazi et al., 2006; Jemal et al., 2007). Diethylnitrosamine (DEN) is a well known hepatocarcinogen present in tobacco smoke, water, preserved meats, curd, fried meals, agricultural chemicals, cosmetics and pharmaceutical products (Sullivan et al., 1991; Brown, 1999; Farazi et al., 2006; Jemal et al., 2009). It is a representative chemical of the family of carcinogenic N-nitroso compounds which induces single strand breaks in DNA of liver cells, also induces changes in several enzymes which are involved in DNA repair and is generally used to induce hepatocellular carcinoma in experimental animals (Bhosale et al., 2002; Mihae et al., 2010).

Historically, only 10–20% of primary HCCs are resectable at the time of diagnosis (Fong et al., 2001). HCC is associated with chronic liver injury, primarily the chronic viral hepatitis and alcoholic liver disease (Bruix et al., 2004; Llovet et al., 2004). The highest incidence of HCC is found in regions where the hepatitis B (HBV) and hepatitis C virus (HCV) are endemic. About 80% of people with hepatocellular carcinomas have cirrhosis (Kamel and Bluemke, 2002). Accumulating reports suggest the existence of high degree of
alterations in p53 in HCC and focus on its role in the pathogenesis, treatment and prognosis of HCC (Attallah et al., 2003; Marotta et al., 2004; Guzman et al., 2005; Bai et al., 2006; Breuhahn et al., 2006). In the regions with dietary exposure to aflatoxin B1 (AFB1), specific mutation at codon-249 of the p53 gene has been detected in 30-47% of HCC patients (Kimbi et al., 2005). Other HCC-agents implicated in the development of HCC in correlation with p53, include nutrition (Mehta, 1995), alcohol consumption (Staib et al., 2003), vinyl chloride exposure (Weihrauch et al., 2000), oral infection (Sarin et al., 2001), oral contraceptive use (Benedetti et al., 1996), and some trace elements such as selenium (Wei et al., 2001; Irmak et al., 2003).

A number of options for treatment of HCC are currently available. These include surgery, systemic chemotherapy, loco-regional treatment and symptomatic relief. Of all these, surgery is proven to be more beneficial. The feasibility of liver resections is very low and the reasons for this include extensive local diseases as well as presence of extra-hepatic diseases. Much research is focused on development of better methods to detect HCC at an early stage so as to allow curative surgery. Although, in the long-term, primary prevention strategies such as wide scale hepatitis B vaccination and reduction of aflatoxin contamination are likely to yield significant benefit, it is still necessary to target treatments towards those patients at risk. Proteomic analysis of HCC has opened new vista for the identification of novel diagnostic biomarker and disease specific associated proteins that are potential therapeutic targets in the treatment of HCC. At present, the proteomics based biomarker discovery for early detection of cancer is gaining more attention.

Biomarkers are biological molecules that are indicators of physiologic state and also of change during a disease process. The utility of a biomarker lies in its ability to provide an early indication of the disease, to monitor disease progression, to provide ease of detection, and to provide a factor measurable across populations (Lee et al., 2009). The initial draft of the human genome has set the pace for biomarker discovery and provided the impetus for the next level of molecular inquiry, which is represented by functional genomics or proteomics (Chaerkady et al., 2008). Biomarkers play a major role in all aspects of
personalized medicine, not only in early disease detection, but also in outcome prediction and evaluation of therapeutic responses.

Proteomics is an indispensable tool for biomarker(s) discovery that provides the better understanding of cancer progression as well as can help in its early detection. Due to the complex multifactorial nature and heterogeneity of the cancer syndrome it is not detected at early stage (Marrero et al., 2005; Posadas et al., 2005; Pang et al., 2008). To date, no effective treatment is available for advanced cancers, which remain a major cause of morbidity and mortality (Aebersold et al., 2003; Graham et al., 2005; Jie-Feng et al., 2008). Thus, there is an urgent need to unravel novel biomarkers for early detection of liver cancer. It is estimated that 45,000 human genes generate approximately 2,50,000 spliced variant of RNA, which are translated into over 1.5 million proteins as a result of post-translational processing and modification (Mann et al., 1993; Anderson et al., 2001; Aravalli et al., 2008; Cheung et al., 2008; Amelie et al., 2009). Major functional information of the cancer-associated genes resides in the proteome. Proteomics has revolutionized the way one looks at and deals with wide variety of biomarker(s) discovery and drug development. Routine cancer diagnosis is based on microscopical examinations of morphological alterations of cells and tissues. In many instances, the distinction between benign and malignant lesions is clear. However, in a substantial number of cases, diagnosis may be ambiguous and the prognosis of the disease is difficult to determine. Altered protein expression due to disease offers the basis for detection of biomarkers and drug targets through analyzing the protein expression profiles with the help of proteins present in body fluids including serum, spinal fluid, urine etc (Patton, 2002; Schneider et al., 2005). A major challenge is to identify the constituents of the human proteome in order to understand the human genome (Southan, 2004; Chantragan et al., 2010). HCC is one of the most common cancers worldwide and a major cause of death in patients with cirrhosis (Bruix et al., 2004). HCC usually occurs as a complication of chronic liver disease and most often arises in patients with HBV- or HCV-related cirrhosis (Reinders et al., 2004; Sangiovanni et al., 2006). Early diagnosis of HCC improves the chance of regression and survival rate. Improvements in imaging modalities have
increased sensitivity, but at the cost of specificity. Currently available biomarker(s) lack adequate sensitivity or specificity. There is no reliable screening blood test for liver cancer. The most widely used biochemical blood test for HCC is serum level of Alpha-feto protein. Alpha-feto protein (AFP) is made by immature liver cells in the foetus and it is elevated in less than 60% of patients. Increased levels of AFP are common in patients with chronic hepatitis also thus decreasing the utility of this test for surveillance purposes (Cheung et al., 2008). Des-gamma-carboxyprothrombin (DCP), also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II), is an abnormal form of the coagulation protein, prothrombin. A 1984 study first described the use of DCP as a marker of HCC; it was detected in 95% of HCC patients. The DCP level did not change with the administration of vitamin K, suggesting a defect in gamma-carboxylation activity rather than vitamin K deficiency (Christopher et al., 2009). A number of subsequent studies have since confirmed this phenomenon. However, in a comparison of various HCC tumor markers, DCP was found to be least sensitive to risk factors for HCC (such as cirrhosis) (Cheung et al., 2008). Further, DCP is not superior to AFP in early detection of HCC in patients with advanced hepatitis C and neither AFP or DCP alone, nor the combination of AFP and DCP was sufficiently accurate to be used for HCC surveillance. However, the combination of both markers enhanced the sensitivity, indicating that these two markers are complementary. Due to poor prognosis and high recurrence of HCC, there is an urgent need for development of novel chemopreventive strategies that selectively target key molecules aberrantly expressed during HCC to improve patient survival (Eberini et al., 2000).

Proteomic approaches will also play an important role in the discovery of biomarkers. Ultimately such biomarkers would aid clinicians in diagnosing liver cancer in the early stages, eliminating the need for liver biopsy and allowing early treatment, thereby preventing the progression of fibrosis.

In view of this, the present study was undertaken to unravel novel biomarkers for early detection of liver cancer using proteomic approach including one-dimensional electrophoresis (1D), two-dimensional electrophoresis (2DE), liquid chromatography mass spectrometry and western blot analysis.
OBJECTIVES

The aim of the study is to analyse the proteins that are differentially expressed during liver cancer progression and assess their potential for the development of biomarker(s) for early detection of HCC. Following are the specific objectives:

❖ Development of a animal model for the study of liver cancers (hepatocellular carcinoma).
❖ Analysis of total liver protein from normal and tumor bearing animal.
❖ Identification and characterization of differentially expressed protein.
❖ Evaluation of differentially expressed protein and biomarkers for early detection of hepatocellular carcinoma.