Identification of Differential Protein Expression in Hepatocellular Carcinoma Induced *Wistar Albino* Rats by 2D Electrophoresis and MALDI-TOF–MS Analysis

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Abstract Hepato cellular carcinoma (HCC) is a type of malignant tumor. To investigate the proteins in cancer molecular mechanism and its role in HCC, we have used proteomic tools such as 2DE and MALDI-TOF–MS. Our investigation reveals that, plasma α -fetoprotein and carcinoembryonic antigen levels were elevated in DEN induced rats and gradually decreased after the treatment with 1,3BPMU. 2DE and MALDI-TOF–MS tool offers to identify the up and down regulation of proteins in HCC. Proteomic study reveals that, five differentially expressed proteins were identified in DEN induced rats and 1,3BPMU treated rats i.e. three up regulated protein such as T kininogen, NDPKB, PRMT1 (DEN induced rats), RGS19 and PAF (1,3BPMU treated rats) in 3BPMU treated rats, activation of transcription of a single gene from multiple promoters provides flexibility in the controlled gene expression. The regulations of hepatocyte stimulating factor were slow down the proliferation of hepatic cell and uncontrolled hepatic cell growth and also molecular signals strongly argue for a patho-physiological role in liver metastasis to control the cell aggression. This indicates that, anti cancer property of 1,3BPMU can be used as potent anti cancer agent. The present study also shows the proteomic approach helps to elucidate the tumor maker as well as regulatory marker proteins in HCC.

Keywords Anti cancer activity · Hepato-cellular carcinoma · Proteome · 2D Electrophoresis · MALDI-TOF–MS · Differential protein expression

Introduction

Hepato-cellular carcinoma (HCC) is a type of liver cancer. This type of cancer occurs more often in men than women, it is not the same as metastatic liver cancer, which starts from another organ (such as the breast or colon) and spreads to the liver. Several major risks related to HCC occurrence such as hepatitis B and/or hepatitis C virus infection, aflatoxin B1 exposure, alcohol consumption and genetic defects [1]. To find the molecular basis of hepato-carcinogenesis, there has been recently intense interest in the study of protein expression, an approach known as differential protein expression analysis [2]. Proteins perform and regulate most biological and biochemical functions. Analysis of the whole proteome may provide functional information by protein and genome expression studies. Since these proteins are potential diagnostic tools or leads for drug and also has great potential in the modern drug discovery process. Differential expressions of the proteome in HBV- and HCV-associated with HCC were investigated to identify any useful biomarkers indicating virus-specific hepato-carcinogenesis [3].

Proteomics is the term used for exhaustive analysis of protein structure and function in an organ or tissue. The levels of gene expression and protein production are not necessarily proportional, and protein activity is frequently regulated by post translational modifications such as Phosphorylation [4]. Proteomic is useful for elucidation of the pathology and discovery of disease markers for HCC and chronic liver diseases. Comparison of HCC and normal

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liver tissues by using dimensional polyacrylamide gel electrophoresis (2DE) and matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF-MS) may reveals to evaluate the protein expression and also to identify the proteins involved in HCC and its function in cancer molecular mechanism [5].

2DE and MALDI-TOF-MS enable to evaluate the disease associated proteins and also many researcher used to find out difference in protein expression [6–10]. 2DE is used to separate the proteins based on charges in the first dimension and molecular mass separated by second dimension [11]. 2DE based proteomic research in liver cancer have been conducted in tissue of HCC liver, cell lines of HCC and sera at cellular level to identify the protein and its expression in liver cirrhosis, drug toxicity and carcinogenesis state [12–16]. Proteomics-based clinical studies have been shown to be promising strategies for the discovery of novel biomarkers and its role. Many chemotherapeutic drugs have been used for the treatment of HCC and it has own implicated therapeutic effect. Alendronate is pyrophosphate analogues which, one of the potential anticancer drug [17].

The present study is design to investigate (a) up and down regulation protein profile (b) to find out differential protein expression and (c) to elucidate the tumor maker as well as regulatory marker proteins associated with HCC by using 2DE and MALDI-TOF-MS in DEN induced rats as well as in 1,3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl]-urea (1,3BPMU) treated rats.

Materials and Methods

Sources of Chemicals and Materials

DEN and Phenobarbital were purchased from Sigma Aldrich, USA. 1,3BPMU were synthesized at the Department of chemistry, National Institute of Technology (NIT), Tiruchirappalli, Tamilnadu, India. The following chemicals and materials required for 2D electrophoresis were obtained from Sigma-Aldrich (St. Louis, MO); glycine, ammonium persulphate (APS), acrylamide/bisacrylamide (solution), glycerol and tetramethylethylenediamine (TEMED). Tris were provided from Roche (Roche Chemicals, Basel, Switzerland). DTT was purchased from Crystal Chem (Downers Grove, Illinois, USA). *Ampholyte* (3–10) was purchased from Fluka (St. Louis, MO). *Protein marker* was obtained Fermentas (St. Leon-Rot, Germany). For mass spectrometry, the following substance was used: trifluoroacetic acid for spectroscopy (Merck, Darmstadt, Germany), water and acetonitrile for MS (Fisher Scientific, Schwerte, Germany), ammonium bicarbonate for MS (St. Louis, MO) and *a*-cyano-4-hydroxycinnamic acid (Bruker

Daltonik, Bremen, Germany). Unless otherwise stated, all other chemicals used were from Sigma-Aldrich (St. Louis, MO).

Synthesis of Mannich Base

As a complement we have received this organic compound from Dr. D. Tamilvendan, Department of Chemistry, National Institute of Technology, and Trichy. His procedure, Urea (6 g, 0.1 M), 2-naphthol (14 g, 0.1 M), and benzaldehyde (10 ml, 0.1 M) were taken in an equimolar ratio. A concentrated aqueous solution of urea and 2-naphthol was prepared. Benzaldehyde was added in drops with continuous stirring of the solution. The mixture first becomes oily and then slowly turned into a white crystalline mass, which was separated by suction filtration and washed several time with diethyl ether, dried and re-crystallized using ethanol by the slow evaporation method [18].

Experimental Animals

Male *Wister albino* rats (Rat: *Rattus norvegicus*) of the same age group with body weight 150–170 gm was selected for all the experiments. The animals were housed in polypropylene cages at an ambient temperature of 25 °C and 40–55 % relative humidity with 12 h each of dark and light cycle. The rats were fed with pellet diet and water ad libitum. All animal experiments were duly approved by the Institutional Animal Ethics Committee [743/03/abc/CPCSEA dt: 03.03.2003/Ph.D/BT04/2013-14] and followed CPCSEA guidelines.

Experimental Design

The rats were divided into four groups; each group consisted of six animals analyzed for a total experimental period of 16 weeks as follows: Group A ($n = 6$): Control rats fed with standard diet with ad libitum of water. Group B ($n = 6$): 1,3 BPMU was given orally to rats (50 mg/kg/bw/day/orally) at 24-h intervals for 16 weeks. Group C ($n = 6$): Rats were induced HCC with DEN using single intraperitoneal (ip) injection (200 mg/kg/bw/) as a cancer inducing agent followed by administration of Phenobarbital (250 mg/kg/bw/day/orally) for two weeks as a cancer promoting agent [19]. Group D ($n = 6$): Rats were induced HCC with DEN using single intraperitoneal (ip) injection (200 mg/kg/bw/) as a cancer inducing agent followed by administration of Phenobarbital (250 mg/kg/bw/day/orally) for 2 weeks as a cancer promoting agent. After 2 weeks, the animals were treated with 1,3BPMU (50 mg/kg/bw/day/orally) for the next 14 weeks [20].

After 16 weeks, experimental rats ($n = 6$ per group) were anesthetized with sodium pentothal after overnight

fasting. Blood was collected from tail vein immediately and animals were sacrificed by cervical dislocation. Liver samples were also collected for histological studies and biochemical analysis. The liver was excised immediately and rinsed in ice cold saline. A portion of the liver was homogenized in 0.1 M Tris buffer, pH7.4 and used for the assays. The serum tumor markers such as α -fetoprotein (AFP) and carcinoembryonic antigen (CEA) were estimated by chem-luminescent immunoassay [21].

Sample Preparation for 2D Electrophoresis

Trichloroacetic acid/acetone precipitation method was followed to prepare the protein from the experimental rat liver with some modification Damerval protocol [22]. Fresh liver tissue were cut into small pieces and homogenized with PBS by mechanically, then it centrifuged in 10,000 rpm at 4 °C for 10 min. The concentrations of protein were determined by Bradford [23]. 500 μ l of supernatant was collected along with equal volume of ice cold acetone 10 % TCA was added to that and incubated for overnight at –20 °C. The homogenate was centrifuged at 10,000 rpm, 4 °C for 15 min, the supernatant was removed and the pellet was rinsed twice with ice cold acetone. The pellet was air dried and resuspended by 85 mM Tris, pH6.8, 0.2 % SDS contains lysis buffer.

Two Dimensional Electrophoresis (2D PAGE)

The method used is based on the one described by O'Farrell [24], but with some modifications. Iso-electric focusing (IEF) electrophoresis was used to separate the proteins according to isoelectric point (pI) in the first dimension. IEF gels were made in glass tubing (160 $\times$ 3 mm² inside diameter) and contained 10.3 g urea, 7.125 ml distilled water, 2.44 ml acrylamide (28.38 % acrylamide, 1.62 % bis-acrylamide), 0.750 ml carrier ampholytes 3/10, 0.375 ml NP-40, 34.625 μ l ammonium persulphate (10 %) and 12.5 μ l TEMED. After half an hour's polymerisation, a pre-run for focusing the ampholytes was performed by loading 30 μ l lysis solution (9.8 M urea, 2 % NP-40 (10 % in distilled water), 2 % carrier ampholytes 8/10, 25mMDTT] and over 30 μ l overlay solution (8Murea, 1 % carrier ampholytes 8/10, 5 % NP-40, 25 mM DTT). Upper running buffer (20 mM NaOH) was degassed for 10 min, but the lower one (10 mM H₃PO₄) was not. The electrophoretic conditions of the rod gels during the IEF were 200 V for 15 min, 300 V for 30 min and 400 V for 1 h. The solution was removed from the upper tank. Meanwhile, the sample (350 μ g) was prepared by adding lysis solution in a 1:2 proportion and heating for 2 min at 100 °C. After loading, 30 μ l of overlay solution was added above every sample, which was

then run at 400 V for 16 h. After focusing, IEF gels were maintained in equilibrating buffer (6 M urea, 75 mM Tris–HCl pH 8.8

MALDI-TOF–MS

Gel pieces of 1.5 mm in diameter were excised manually from the 1-mm thick gels and washed for 30 min at room temperature under vigorous shaking with 400 μ l of 10 mM ammonium bicarbonate solution containing 50 % (v/v) acetonitrile. After removing the supernatant, gel pieces were dried for 15 min in a vacuum concentrator. For digestion, 5 μ l trypsin solutions (Sequencing grade modified trypsin, Promega, Madison and 10 ng/ μ l in 5 mM ammonium bicarbonate; 5 % acetonitrile) were added to each sample. After incubation for 5 h at 37 °C, the reaction was stopped by adding 1 μ l of 1 % TFA. For better extraction of peptides, the samples were stored overnight at 5 °C. Without further purification, 1 μ l supernatant was mixed with 2 μ l of matrix solution (5 mg *a*-cyano-4-hydroxycinnamic acid in 40 % [v/v] acetone, 50 % [v/v] acetonitrile, 9.9 % [v/v] water and 0.1 % [w/v] TFA in water). From this mix, 1 μ l was deposited onto the MALDI target. Tryptic peptides were analyzed with a MALDI-TOF mass spectrometer (S, Germany) in positive mode. Background ions from trypsin autolysis and contamination by keratins were removed from mass lists. Protein identification was performed by searching for *Rattus* proteins in the latest version of the NCBI database using the Mascot search engine [26]. The following parameters were applied: monoisotopic mass accuracy, 100 ppm; missed cleavages, 1; allowed variable modifications, oxidation (Met) and fixed modification, Carbamidomethyl (C). Fragmentation of selected peptides was measured using the PSD mode. The mass lists were used for MS/MS search applying the database and search engine as mentioned above.

Statistical Analysis

The average numbers of 2D protein spots between the gel are expressed as mean $\pm$ standard error of the mean (SEM). Statistical comparisons were performed by oneway analysis of variance (ANOVA) by using SPSS 14 version statistics software. The results were considered statistically significant if the *P* values were 0.05 or less.

Results

Figure 1 denotes the content of tumor marker proteins in control and experimental group of rats. The α -fetoprotein (AFP) and carcino embryonic antigen marker protein levels were elevated in DEN induced rats as compared to the

Fig. 1 Level of CEA and AFP in control and experimental group of rats. Values are mean $\pm$ SD of six animals. Statistical significance $P < 0.05$

control rats. However, Cancer bearing rats treated with 1,3BPMU reduced these markers as compared with DEN induced rats. There are no variations observed in 1,3BPMU alone treated rats. Proteomic analysis of experimental group of rat liver was performed by two dimensional electrophoresis and MALDI-TOF-MS. The tissues of liver protein were separated and average 350 μ g of total protein was used for 2DE. Representative Coomassie blue stained 2DE gels of liver samples from controls and 1,3BPMU alone, DEN-induced and DEN induced cum 1,3 BPMU treated group are shown in Fig. 2. Each set of animal plasma (six samples from each groups) were processed in parallel through protein extraction (TCA/acetone method), 2DE, and staining to avoid differences from sample handling. The resulting six gel images (each group) were analyzed and evaluated as one match set with the 2D software, i.e., Melanie Viewer 7 ($P < 0.05$). A total of 480 protein spots were identified in all the groups, out of which three up regulated proteins in DEN induced rats and two up regulated proteins in 1,3BPMU treated rats. Differentially expressed protein spots in 2DE gels are indicated by arrows in Fig. 2 ($P < 0.05$).

We have selected five reliable protein spots for MALDI protein identification (three spots from DEN induced group and two spots from 1,3BPMU treated group). The expressed proteins spots were digested and the mass of the tryptic fragments were determined using MALDI-TOF-MS (Fig. 3). Each spots were subjected to a database analysis using mascot with SwissProt (<http://www.matrixscience.com/>). The mascot data analysis reveals that, spot 1 was identified as T-kininogen-2, the molecular weight is 47704 Da, spot 2 was identified as protein arginine *N*-methyltransferase-1 with the molecular weight of 40522.4 Da, spot 3 was identified as Nucleoside diphosphate kinase-B with the molecular weight of 17282.9 Da,

spot 4 was identified as platelet-activating factor acetylhydrolase with the molecular weight of 46670 Da, spot 5 was identified as regulator of G-protein signaling-19 with the molecular weight of 27644.5 Da. The obtained mass signal results and protein access codes, peptide sequence coverage, pI, M.WT, instability index (II), aliphatic index (AI), and grand average of hydropathicity are shown in Table 1. Numbers of spots, average correlation coefficient, and mean SD for spot intensities from each of the sets of gels are shown in Table 2 ($P < 0.05$). The download date of the SwissProt database was 24 May 2014 at 06:39:06 GMT. SwissProt database sizes of the sequence database were [SwissProt: 2014_05 (545388 sequences; 193948795 residues) (Rattus: 7911 sequences)].

Discussion

Alpha fetoprotein (AFP), a tumor associated fetal protein, has long been employed as a serum fetal tumor marker to monitor disease progression. Carcinoembryonic antigen (CEA) is a another tumor marker found in many types of cells and also associated with tumors and developing fetus. Alpha-fetoprotein (AFP) is a glycoprotein that is produced in early fetal life by the liver and by a variety of tumors including hepato-cellular carcinoma. AFP and CEA levels greater than control are correlated with the tumor and also it's confirm that HCC [25]. The treatment with 1,3BPMU shows that, significant reduction AFP and CEA levels in plasma. Our result suggests that, 1,3BPMU is a potent anti cancer agent. T kininogen, a major acute-phase protein in the rat, belongs to a multigene family of four genes-two for highly homologous T kininogens (Ti and T2 kininogen), one for K kininogen that corresponds to the kininogen in other mammals, and a structurally related pseudogene

Fig. 2 Reference two-dimensional electrophoretic patterns of rat liver proteins obtained from a control (a), 1,3 BPMU alone treated (b), DEN induced (c) DEN induced and 1,3 BPMU treated (d)

[26, 27]. T kininogen expression is more specific to liver genes, related to acute phase genes and drastically altered when body homeostasis is disturbed by tissue injury or infection [28]. In addition to the constitutive liver-specific transcription factors, regulation of these genes also involves in factors that are induced or activated by inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor. Acute phase response is related to microbial or viral disturbances of homeostasis due to infection, tissue injury, neoplastic growth or disorder in immunological levels which happened by specific proteins to restoring the disturbed pathological homeostasis [29]. Sometime T-kininogen 2 is an acute phase extra cellular protein. IL-6 is a potent inducer of acute phase proteins synthesis in rat hepatoma cells during HCC by various factors such as environmental pollution, chemical caecinogens, HBV and HBC. In adult rat under HCC condition disturbances of homeostasis occurs by free radicals. Due to above

disturbances infection or tissue injury occur lead to biological activity with moderate to strong correlation with tissue damage [30]. This inflammation leads to formation and activation of leukocytes, fibroblast, and endothelial cell damages either by more activation or suppression. These changes cause and release IL-6, IL-1, TNF and interferon. Among these IL-6 has the capacity to release acute phase proteins in liver tissues such as β -fibrinogen, α -antichymotrypsin, α -acid macroglobulin and cysteine protease inhibitors (T-Kininogen 2) in rat hepatocytes along with hepatocyte stimulating factor III [31]. The above mechanism leads to occurrence of HCC. As per our investigation DEN causes cancer by hepatocyte stimulating factor (T-Kininogen 2) which leads to HCC. Our result also supports the above statement based on up-regulation of T-Kininogen 2 in DEN induced leads to oxidative damage and oxidative stress and hepatocyte stimulating factor. The treatment with 1,3BPMU shows that, down-regulation of

Fig. 3 MALDI-TOF-MS mass spectrum of the tryptic digest of spots obtained in the linear TOF-MS for highest resolution and mass accuracy

T-Kininogen 2 in 1,3BPMU treated rats. The down regulations of hepatocyte stimulating factor were slow down the proliferation hepatic cell and uncontrolled hepatic growth. This indicates that, anti cancer activity of 1,3BPMU and also 1,3BPMU can be used as potent anti cancer agent.

Protein methylation is one of the processes in post translational modification catalysed by specific of methyl transferases. About 2 % arginine residues are methylated in rat liver nuclei via post translational modification. Protein arginine methyl transeferase 1 (PRMT 1) is an intra cellular enzyme. It catalyse the addition of one or two methyl group to guanodino nitrogen atoms of arginine resulting mono-methylarginine (MMA), symmetric methyl arginine (SMA) and asymmetric methyl arginine (ASMA) [30]. Protein arginine methylation is an abundant modification that has been implicated in signal transduction, gene transcription, DNA repair and mRNA splicing [32]. Studies have only recently linked this modification to carcinogenesis and metastasis. The expression of some genes encoding PRMTs may be altered in certain cancers. The nuclear receptor co-activators PRMT1 and CARM1 were over

expressed in breast cancers, liver cancer and prostate cancers. The over expression of PRMT1 enhanced messenger RNA level of Fox O1 which trigger the gluconeogenesis, as a result higher production of glucose in primary hepatocytes by phosphorylation of Fox O1 cancerous cells in liver tissues [33, 34]. Our result also supports the above statement based on up-regulation of PRMT1 in DEN induced leads to uncontrolled methylation cause conformational changes in proteins. The treatment with 1,3BPMU shows that, down-regulate the synthesis or/and deactivation of PRMT1 in 1,3BPMU treated rats were leads to abolish the more methylation process in post translational modification. This indicates that, anti cancer activity of 1,3BPMU and also it can be used as potent anti cancer agent.

Nucleoside di-phosphate kinase B (NDPKB) which catalyzes the exchange of phosphate groups between nucleoside di-phosphates. NDPKB is highly homologous to nm23 gene product. It is possible both nm23 gene product and NDPKB may be active in the progression and differentiation of tumor cell. NDPK has been detected in the membrane, cytoplasmic and nuclear fractions of cells [35, 36]. Generally NDPKB is the most important component of the enzymatic pathway for

Table 1 List of identified in MALDI-TOF-MS

Accn. no.	Name of the protein	Score	Estimate M.W (Da)	PI	II	AI	GRAVY
P08932	T-Kininogen 2	22	47704.1	5.94	33.13	73.26	−0.416
Q63009	Protein arginine <i>N</i> -methyltransferase 1	19	40522.4	5.37	41.69	83.09	−0.207
P19804	Nucleoside diphosphate kinase B	56	17282.9	6.92	34.16	86.51	−0.270
Q4L0E8	Regulator of G-protein signaling 19	27	27644.5	5.31	73.49	59.46	−0.642
P63004	Platelet-activating factor acetylhydrolase	31	46670.0	6.97	34.71	70.78	−0.514

Up-regulated proteins of rat liver tissue in DEN induced and DEN induced, 1,3BPMU treated proteins

MW molecular weight, *Da* Dalton, *pI* isoelectric point, *II* instability index, *AI* aliphatic index, *GRAVY* grand average of hydropathicity

Table 2 six samples were selected for each group to create merged images and the resulting mean spot volumes were plotted against each other

Groups	Total Spots	Average correlation con efficient	Mean SD	<i>P</i> value (<i>P</i> < 0.05)
Group I (<i>n</i> = 6)	453	0.90	±17.00	0.041
Group II (<i>n</i> = 6)	461	0.95	±13.00	0.037
Group III (<i>n</i> = 6)	469	0.91	±19.00	0.028
Group IV (<i>n</i> = 6)	472	0.94	±12.00	0.019

As expected this resulted in a straight line ($R^2 = 0.9$). Values are mean $\pm$ SD of six animals. Statistical significance $P < 0.05$

the synthesis of nucleoside tri-phosphate and also NDPKB activity is mainly controlled by hormonal dependent signaling mechanism. Hepatic cell higher proliferative response from free radicals leads to NDPKB activate signaling pathway by means of ATP formation. To produce more ATP, NDPKB activate signaling mechanism support the cell proliferation and cell division [37, 38]. In our study, we found that the expression of NDPKB protein in transient in cancer bearing tissues (DEN induced group). This result suggested, in the early stage of NDPKB protein over expression support the timorous cell division and proliferation through production of more ATP. The treatment with 1,3BPMU shows that, down-regulate the synthesis NDPKB by means of ATP necessity controlled by signaling pathways in 1,3BPMU treated rats and it were slow down the proliferation of hepatic cell and uncontrolled hepatic growth. This indicates that, potential anti cancer activity of 1,3BPMU and it can be used as potent anti cancer agent.

G protein coupled receptors (GPCR) regulates the growth of cells and differentiation of cells by the way of signaling pathways like tyrosine kinase. They were many extra cellular and intra cellular signaling pathways and signal transduction machannism associated with HCC. Deregulation of this pathway may favor to develop HCC. Signals arising from G GPCRs can be terminated by regulators of G protein signaling. Regulator G protein are a multifunctional proteins. It may promote or inhibit cell proliferation and cell differentiation [39]. Among that regulatory G protein signaling 19 (RGS19) (G α -interacting

protein/GAIP) is mainly involved in intra cellular signaling pathways which leads modulate cell proliferation [40]. Ras is an essential component of signal transduction pathway that control cell proliferation, differentiation and survival [41]. The up regulation of RGS19 to induce Nm23 and inhibit Ras activation via phosphorylation of KSR and other pathway also contribute towards Ras suppression especially the activity of C-Jun-N-terminal kinase (JNK). It implicated with tumor suppressor protein, an elevated level expression of RGS 19 by vascular endothelial cells may inhibit the signal transduction by increasing GTPase activity of α subunit [42]. Our result also supports the above statement based on up-regulation of RGS19 in 1,3BPMU treated rats leads to inhibit the signal transduction by increasing GTPase activity of α subunit and in turn controls cell proliferation and cell differentiation. This indicates that, anti cancer activity of 1,3BPMU and also it can be used as potent anti cancer agent.

Platelet-activating factor (PAF) is highly potent phospholipid mediate and it has capable to induce immune regulatory actions [43]. PAF acetylhydrolase act a regulatory enzyme by control the formation and immune regulatory mechanism of PAF [44]. Liver is thus a potentially PAF target since PAF, lyso-PAF, AHA and PAF-R are found in normal liver. Tissue PAF concentrations are physiologically up regulated by a specific PAF acetylhydrolase activity (AHA). The PAF receptor (PAF-R) gene produces 3 different species of mRNA (i.e., transcript 1, transcript 2 and an elongated form of the transcript 2), which generate a unique membrane PAF-R. PAF-R transcripts 1 and 2 are also

named leukocyte type and tissue type, respectively. PAF-R transcripts 2 (both normal size and spliced variant) was found in the tumor tissue as compared with the non tumor one. Several compounds such as tumor necrosis factor transforming growth factor and PAF itself enhance total PAF-R mRNA synthesis in several cell types [45, 46]. Transcription of a single gene from multiple promoters provides additional flexibility in the control of gene expression. It is interesting to note that in liver metastasis of colorectal carcinoma, only PAF-R transcripts 2 (the PAF-R tissue type) are up regulated. Such an up regulation of PAF-R transcripts 2 has been previously reported in human umbilical vascular endothelial cells in response to stress and in endometrial cells in response to estradiol, inflammation mediated by macrophages-recruitment to tissues; differentiation and activation in situ to suppressive cells and restoration of tissue homeostasis [47]. Our result also supports the above statement based on up-regulation of PAF in 3BPMU treated rats leads to activation of transcription of a single gene from multiple promoters provides additional flexibility in the control of gene expression. Whatever molecular signals implicated in these elevated PAF levels strongly argue for a pathophysiologic role of PAF in liver metastasis to control the cell aggression.

Conclusion

In summary, we investigated the feasibility and interest of proteomic analysis in HCC, which gives complete protein profile as well as differential protein expression map. We have identified anti cancer drug-modulated proteins that participate in several cellular responses including proliferation of hepatic cell, uncontrolled hepatic growth, transcription control, signal transduction, protein folding post translational modification and up and down gene expression. Our findings indicate potential markers of HCC that are suitable for early-stage evaluation of disease diagnosis and prognosis. The identified proteins might also represent potential targets for treatment of HCC and associated complication. Proteomic tools such as 2DE and MALDI-TOF-MS highly effective approach to elucidate differential protein expression such as T kininogen, NDPKB, PRMT1 (DEN induced rats), RGS19 and PAF (1,3BPMU treated rats) in 1,3BPMU treated rats, which was one of the factors revealed in the present study, resulting in controlled hepatic cell proliferation and also this strategy could reveals anti cancer activity of 1,3BPMU and also helps to elucidate the tumor maker as well as regulatory marker proteins in HCC. In order to further elucidate the molecular mechanisms involved in 1,3BPMU cytotoxicity a better understanding is needed for the molecular events following 1,3BPMU treatment for HCC.

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Conflict of interest The authors are not having any conflict of interest related to this research paper.

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