RESEARCH ARTICLE

Identification of Histone Deacetylase 1 Protein Complexes in Liver Cancer Cells

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Abstract

Background: Hepatocellular carcinoma is one of the leading causes of mortalities worldwide. The search for new therapeutic targets is of utmost importance for improved treatment. Altered expression of HDAC1 in hepatocellular carcinoma (HCC) and its requirement for liver formation in zebrafish, suggest that it may regulate key events in liver carcinogenesis and organogenesis. However, molecular mechanisms of HDAC1 action in liver carcinogenesis are largely unknown. The present study was conducted to identify HDAC1 interacting proteins in HepG2 cells using modified SH-double-affinity purification coupled with liquid mass spectrophotemetery. Materials and Methods: HepG2 cells were transfected with a construct containing HDAC1 with a C-terminal strepIII-HA tag as bait. Bait proteins were confirmed to be expressed in HepG2 cells by western blotting and purified by double affinity columns and protein complexes for analysis on a Thermo LTQ Orbitrap XL using a C18 nano flow ESI liquid chromatography system. Results: There were 27 proteins which showed novel interactions with HDAC1 identified only in this study, while 14 were among the established interactors. Various subunits of T complex proteins (TCP1) and prefoldin proteins (PFDN) were identified as interacting partners that showed high affinity with HDAC1 in HepG2 cells. Conclusions: The double affinity purification method adopted in this study was very successful in terms of specificity and reproducibility. The novel HDAC1 complex identified in this study could be better therapeutic target for treatment of hepatocellular carcinoma.

Keywords: Histone deacetylase 1 (HDAC1) - double affinity tag purification - hepatocellular carcinoma

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Introduction

The relatively ubiquitous expression pattern of class I histone deacetylases (HDACs) and the fundamental roles HDACs play in regulating chromatin structure, it was believed that class I HDACs would play a general role in embryonic and cancer development. However, recent findings indicated rather tissue specific expression of HDACs (Witt et al., 2009). Moreover, there is increasing evidence that the expression of HDACs was also altered in various cancers (Coradini and Speranza, 2005; Pathil et al., 2006; Venturelli et al., 2007; Gahr et al., 2008).

Most biological processes are mediated via protein complexes. Hence, to understand molecular events either under normal physiological conditions such as organogenesis, or in pathological state such as cancer, it is necessary to identify and characterize the protein complexes involved. Many HDACs exist as components of multiprotein complexes, such as the transcriptional co-repressors mSin3, N-CoR, and SMRT (Wong and Privalsky, 1998; Karagianni and Wong, 2007). The composition of various HDAC complexes is cell type dependent and fluctuates with intra- and intercellular stimuli. The HDAC complexes play key role at multiple levels in gene expression and genome stability (Delcuve et al., 2013).

Hepatocellular carcinoma (HCC) remains a major health problem worldwide as the third cause of cancer-related mortality (Jemal et al., 2011). HDAC1 is over-expressed in prostate, gastric, colon and breast carcinomas (Halkidou et al., 2004; Zhang et al., 2005; Wilson et al., 2006; Nakagawa et al., 2007). HDAC1 and HDAC3 are specifically implicated in hepato-carcinogenesis (Wu et al., 2010; Yang et al., 2010; Quint et al., 2011; Lachenmayer et al., 2012) and hepto-organogenesis as well (Farooq et al., 2008; Noel et al., 2008). Keeping in view the vital role of HDAC1 in hepato-organogenesis and hepato-carcinogenesis, we have tried to fish out the HDAC1 specific interacting proteins in liver cancer chosing HepG2 cells as our liver cancer model.

A modified affinity purification method coupled with mass spectrophotometery (AP-MS) as described by

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(Glatter et al., 2009) was used with little modification in order to identify HDAC1 protein complexes in liver cancer. A total of 598 HDAC1interacting partners were identified which after subtraction of background from mock transfected cells and protein frequency library assessment, resulted in 86 putative interactors. Twenty eight proteins were sorted out after applying stringent filtration criteria. The AP-LCMS strategy adopted in this study resulted identification of 25 novel binders and 3 known interactor for HDAC1. Out of 28 interactors, various subunits of T complex proteins one (TCP1) and prefoldin (PFDN) proteins were identified as high affinity binders of HDAC1 in HepG2 cells. We believe that this is the first study reporting specific HDAC1 interacting proteins in liver cancer cell lines, which will provide further insight into mechanism of HDAC1 regulation in liver cancer.

Materials and Methods

Cell culture

The human HepG2 cells (ATCC HB-8065) were cultured in DMEM medium (Hyclone, Utah, USA), with high glucose and supplemented with 10% FBS and 2 mM L-glutamine. Cells were grown at 37°C in a humidified atmosphere with 5%CO_2.

Molecular cloning of HDAC1

The HDAC1 gene was amplified from IHS1380-97433344 (Open biosystems, Thermo scientific; Rockford, IL 61101, USA) by performing PCR with forward primer 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AAC CAT GGC GCA GAC GCA GGG CAC-3' and reverse complement primer 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT GGC CAA CTT GAC CTC CTC CT-3'

Bait construction

BP-Gateway Reactions were performed to insert the HDAC1 into pDONR221 Entry-Vector (Invitrogen). Subsequently, the HDAC1 gene from the pDONR221 construct were inserted with a LR-Gateway reaction into a Gateway expression vector with a C-terminal strep3-HA tag (pcDNA3.1-SH-C) following the manufacturer protocol. The constructed plasmids are pcDNA3.1-SH-C -C/HDAC1, C-terminal Strep-HA fusion. The bait sequences were verified by sequencing

Expression of HDAC1 in HepG2 cells

In order to express the HDAC1- Strep-HA fusion proteins, HepG2 cells were transfected with Lipofectamine 2000 (Invitrogen). In brief, cells were cultured to 25% confluency the day before transfection. Transfection was performed at 50% confluency and cells were kept in culture for 48h post transfection. Expression of the constructs was monitored with anti-HA antibodies. For control, HepG2 cells were transfected with mock plasmid under the same experimental conditions.

Affinity purification

Following transfection, around 6x10^6 transfected cells were harvested per replicate for mass spectrometrical analysis. The cells were washed twice with phosphate buffer solution (PBS) and pelleted in PBS by centrifugation at 400g. Cell pellets were resuspended in 3ml of a lysis buffer [50 mM HEPES pH 7.5, 150 mM NaCl, 10 mM NaF, 1.5 mM Na-vanadate, 5% (v/v) glycerol, 0.5% IGEPAL-CA-630 (Sigma), protease inhibitors and phosphatase inhibitors (Sigma), 5 ug/ml avidin 600U benzonase (Merk)]. Lysates were incubated for 10 min on ice and then centrifuged for 20 min at 20000g at 4°C. The cleared lysate was applied onto a 150 ul strepTactin resin column (Ito et al., 2001) and washed 5 times with wash buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 10 mM NaF, 1.5mM Na-vanadate, 5% (v/v) glycerol, 0.05 % IGEPAL-CA-630 (Sigma) and eluted with 10mM biotin in wash buffer.

Mass spectrometrical analysis

Elutes from either mock transfected or HDAC1 were prepared in triplicates and filter assisted sample preparation was performed as described before by (Glatter et al., 2009). The samples were analyzed on a Thermo LTQ Orbitrap XL using a C18 nano flow ESI liquid chromatography system with a 5-50% acetonitrile at 1 hour gradient. A Top5 collision induced dissociation method (CID) with wide band activation was used (Olsen et al., 2009). The samples were analyzed using the Maxquant software suite.

Results

Generation of expression constructs and expression in HepG2 cells

In order to identify HDAC1 interacting partners in HepG2 liver cancer cells, a modified double affinity purification method coupled with LCMSMS was used. The overall strategy is depicted in Figure 1.

The cDNA encoding human HDAC1 (IHS1380-97433344) was used from the gateway compatible human orfeome collection (Lamesch et al., 2007) as a resource to generate expression constructs by LR recombination with a destination vector suitable for tetracycline-controlled expression of affinity-tagged bait proteins.

To improve the yield and specificity of the affinity purified protein complexes, a double affinity purification strategy was applied. Protein complexes were isolated through a small double-affinity tag (SH-tag) consisting of a streptavidin-binding peptide and a hemagglutinin (HA) epitope tag. SH-tagged and associated proteins are first bound to an affinity column containing a modified version of streptavidin StrepTactin (Junttila et al., 2005) and then eluted specifically with biotin onto an anti-HA antibody column at low pH. Western blotting showed that only the bait protein (SH-HDAC1) from HepG2 cell extracts bound to the streptavidin column very efficiently (Figure 2A; HDAC1, cleared lysates). Almost none of the protein was trapped on Strep Tactin column from cell lysate prepared from mock transfected cells (Figure 2A; mock) which mean that this binding was very efficient and specific. The purity and yield increased with second affinity step, as it can be seen that no detectable bait protein was left on the

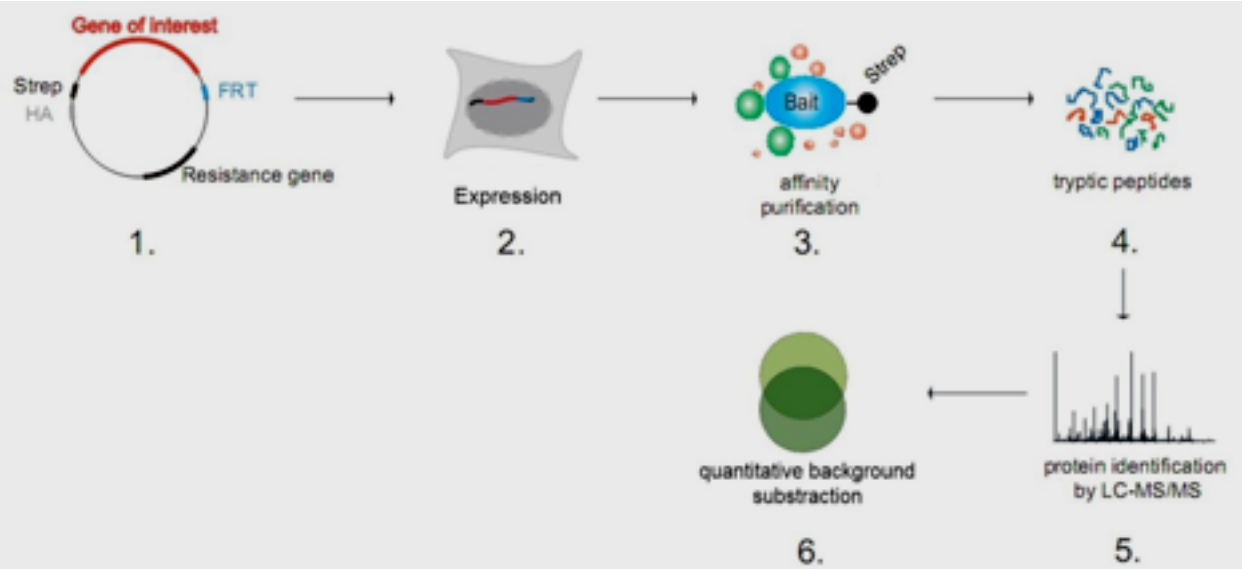

Figure 1. Schematic Representation of the Flow Chart of AP-MS Procedure. Starting from human Gateway orfeome collections, cDNAs of interest are recombined into an expression construct for tetracycline (tet)-inducible expression of strep-hemagglutinin double-tagged (SH) bait proteins. (2) HepG2 cell line was generated using Flp-recombinase-mediated recombination through single FRT sites present in the expression construct. (3) Purification of SH-tagged HDAC1 bait proteins from HepG2 cells using streptavidin sepharose (Strep-Tactin beads). The purification procedure was monitored by immunoblotting using anti-HA antibodies. The complex peptide mixture is separated on a C18 HPLC column and directly analyzed by mass spectrometry (direct LC/MS-MS). (5) The proteomic data obtained was quantitatively filtered by subtracting the background signal intensity from mock trasfected cells

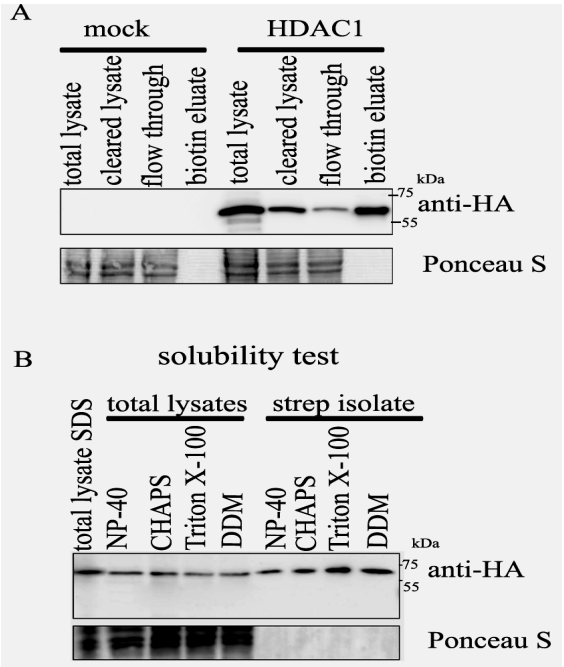

Figure 2. Monitoring SH-double-affinity Purification Efficiency and Solubility Conditions. A) HepG2 cells expressing SH-tagged proteins or vector alone (mock) are lysed and cleared lysate was applied onto a 150ul strepTactin resin column. After several wash steps, purified proteins are released in the presence of 10mM biotin for subsequent immunoaffinity purification using anti-HA agarose. Finally, protein complexes are eluted with 0.2M glycine, pH 2.5, and processed for mass spectrometric analysis. Only the bait protein exclusively bounded to the StrepTactin column not any protein from the mock transfected cells. The specificity and yield increased after second purification step. B) Monitoring of best solubility conditions by immunobloting using Anti HA antibody. Total cell lysate and strep isolates were tested under various buffer conditions and DDM was found to be the optimum buffer for maximum yield

streptavidin beads after the last biotin elution (Figure 2A; flow throw). For the optimal solubility of affinity purified proteins, the most suitable buffer conditions were also tested. DDM (dodecyl-β-d-maltoside) buffer was found to be the best to get the maximum yield and solubility of the proteins (Figure 2B; DDM).

HDAC1 affinity purification and mass spectrometry (HDAC1–AP-MS)

Affinity purification and mass spectrometrical analysis of HDAC1 transiently expressed in HepG2 cells were performed with three biological replicates for quantitative mass spectrometrical analysis. Following tryptic digest, the samples were desalted by a reversed-phase chromatography and directly loaded on a reversed-phase HPLC column attached to an MS instrument for peptide separation and MS analysis. The variations of the biological replicates A, B and C were below than 10% (Figure 3).

The background filtering of HDAC1 affinity purification with mock transfected cells was done to get the specific interactors for HDAC1. In total, 598 proteins were obtained by mass spectrometry, which after subtraction of mock transfected cell background and protein frequency library assessment resulted in 86 putative interactors. A network for of HDAC1 interactors is shown in Figure 5. The previously found sub interactions are shown in red whereas novel interactors found in this study are shown in blue.

Analysis of protein interaction data

We further sorted the obtained data from AP-MS analysis and put very stringent filtering criteria to identify only those proteins having high affinity with HDAC1 in HepG2 cells. All those proteins which had signal intensity ratio of HDAC1/control >10 and having less than 5 unique

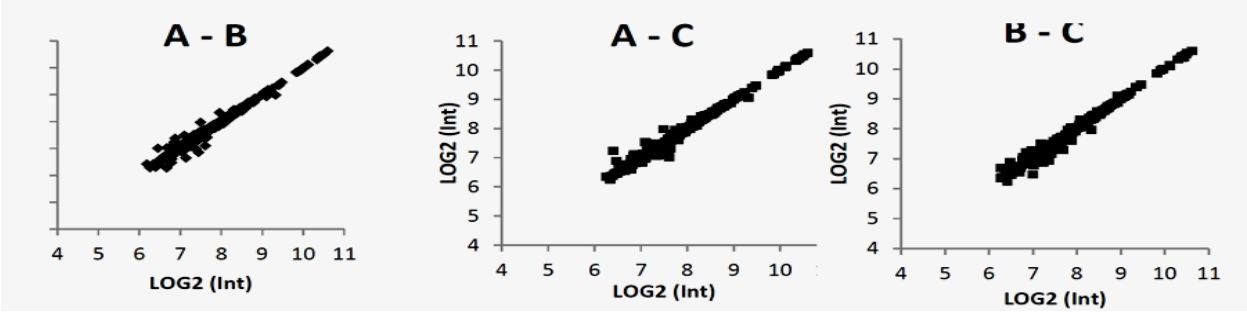

Figure 3. Analysis of HDAC 1 AP-MS Replicate Variation Biological Replicates A, B and C. Pearson correlation between replicates>0.9

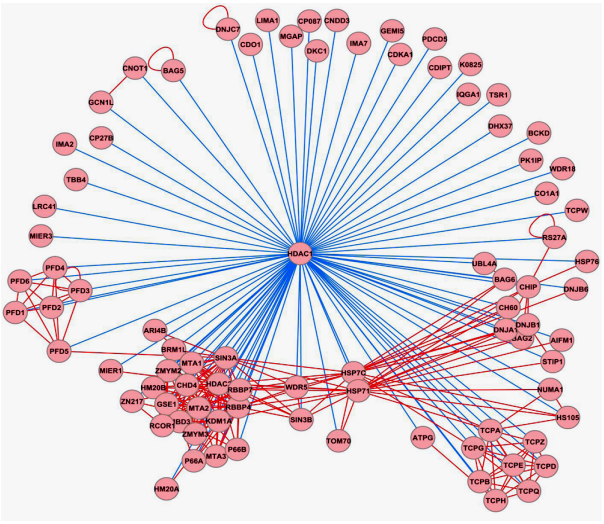

Figure 4. HDAC1 Interactor's Network Experimentally Determined Interactions Displayed in Blue, Curated with Sub Interactions of Interactors Red (source: PINA)

peptides per protein were removed. Only fourty one (41) proteins could pass these filtration criteria. Searching the online protein interaction data bases like PINA (http://cbg.garvan.unsw.edu.au/pina/home.do), Biogrid (http://thebiogrid.org), STRING (http://string-db.org/), resulted only 14 proteins (34%) as known interactors, while twenty seven (27) proteins were identified as novel interactor of HDAC1 in this study. The list of the novel and known interactors are given in Table 1 and 2 respectively. The data is sorted on descending order based on the signal intensities ratios of HDAC1/control.

T complex Protein 1 subunit gamma (TCPG), subunit eta (TCPH), subunit epsilon (TCPE), subunit theta (TCPQ), subunit beta (TCPB) and subunit delta (TCPD) were identified showing highest affinity with HDAC1 in HepG2 cells with maximum sequence coverage of the protein and unique peptides per protein. Another family of proteins which showed specific interaction with HDAC1 in HepG2 cells was prefoldin proteins (PFDN).

Table 1. List of Proteins Identified as Novel Interactors of HDAC1 in HepG2 Cells

Protein ID	Protein Name	Protein Descriptions	Unique Peptides	HDAC1 / control
Q9UHV9	PFD2	Prefoldin subunit 2	6	913.4387453
O15212	PFD6	Prefoldin subunit 6	11	398.5077163
P50991	TCPD	T-complex protein 1 subunit delta	39	316.9847845
P49368	TCPG	T-complex protein 1 subunit gamma	47	297.0902584
P48643	TCPE	T-complex protein 1 subunit epsilon	45	289.4704404
P50990	TCPQ	T-complex protein 1 subunit theta	45	250.8151267
P61758	PFD3	Prefoldin subunit 3	13	227.5353558
P78371	TCPB	T-complex protein 1 subunit beta	44	209.0983151
Q14687	GSE1	Isoform 2 of Genetic suppressor element 1	13	201.8496619
P40227	TCPZ	T-complex protein 1 subunit zeta	25	178.0207926
Q9UL15-2;Q9UL15	BAG5	BAG family molecular chaperone regulator 5	5	155.4083491
Q8WXI9	P66B	Transcriptional repressor p66-beta	11	154.0346968
O60925	PFD1	Prefoldin subunit 1	7	135.30425
Q99832	TCPH	T-complex protein 1 subunit eta	47	96.25238206
Q9NQP4	PFD4	Prefoldin subunit 4	6	64.86791954
O14737	PDCD5	Programmed cell death protein 5	5	55.69882139
O95816	BAG2	BAG family molecular chaperone regulator 2	15	48.29791702
P46379-3;P46379;P46379-2	BAG6	Isoform 2 of Large proline-rich protein BAG6	10	43.36335649
P31689	DNJA1	DnaJ homolog subfamily A member 1	15	32.80229279
Q99615	DNJC7	DnaJ homolog subfamily C member 7	6	31.98063884
Q9UNE7;Q9UNE7-2	CHIP	Isoform 2 of E3 ubiquitin-protein ligase CHIP	12	30.87276787
P10809	CH60	60 kDa heat shock protein, mitochondrial	39	27.15296062
P11142;P11142-2	HSP7C	Isoform 2 of Heat shock cognate 71 kDa protein	27	22.73665955
P31948	STIP1	Stress-induced-phosphoprotein 1	31	21.35271054
O95831;O95831-3;O95831-2	AIFM1	Isoform 2 of Apoptosis-i	21	20.30679201
P52292	IMA2	Importin subunit alpha-2	6	12.23555406
Q92598 -3	HS105	Isoform 3 of Heat shock protein 105 kDa	10	11.47808353

Table 2. Known Interactors of HDAC1

Protein ID	Protein Name	Protein Descriptions	Unique Peptides	Sequence Coverage [%]	HDAC1 / control	Reference
Q99471	PFDN5	Prefoldin subunit 5	9	71.4	1408.083531	(Satou et al., 2001)
O95983;O95983-2	MBD3	Isoform 2 of Methyl -CpG-binding domain protein 3	8	30.9	175.0214885	(Humphrey et al., 2001)
P17987	TCPA	T-complex protein 1 subunit alpha	38	83.1	166.2180566	(Terhune et al., 2010)
Q9UKL0	RCOR1	REST corepressor 1	9	38.4	137.4765199	(Lin et al., 2012)
Q9UBW7	ZMYM2	Zinc finger MYM-type protein 2	23	19.5	122.8527319	(Gocke and Yu, 2008)
O60341;O60341-2	KDM1A	Isoform 2 of Lysine-specific histone demethylase 1A	38	62.1	119.9939405	(Shi et al., 2005)
Q13330;Q13330-2	MTA1	Isoform Short of Metastasis -associated protein MTA1	6	19.6	75.4562506	(Xue et al., 1998, Yao and Yang, 2003, Terhune et al., 2010)
Q09028;Q09028-3;Q09028-2;Q09028-4	RBBP4	Isoform 2 of Histone-binding protein RBBP4	9	53.9	63.93772758	(Cui et al., 2011; Havugimana et al., 2012)
Q16576	RBBP7	Histone-binding protein RBBP7	11	61.2	58.55048001	(Havugimana et al., 2012)
Q96ST3	SIN3A	Paired amphipathic helix protein Sin3a	29	33.1	55.86089009	(Fleischer et al., 2003)
O94776	MTA2	Metastasis-associated protein MTA2	21	46	54.79709158	(Banach-Orlowska et al., 2009, Wang et al., 2009, Havugimana et al., 2012, Lin et al., 2012)
Q14839-2;Q14839	CHD4	Chromodomain-helicase -DNA-binding protein 4	35	21.9	45.07013133	(Zhang et al., 1998)
P08107	HSP71	Heat shock 70 kDa protein 1A/1B	23	77.7	24.70434292	(Johnson et al., 2002)
Q92769	HDAC2	Histone deacetylase 2	7	34.8	22.91795423	(Lin et al., 2012)

Discussion

Hepatocelluar carcinoma is the most common type of liver cancer, and also is a leading cause of death in Asian population (worldwide cancer statistics, cancer research UK 2012, http://www.cancerresearchuk.org/cancer-info/cancerstats/world/cancer-worldwide-the-global-picture). The role of epigenetic events such as methylation and acteylation in variety of cancers is well known. Very little is known about the possible contribution of HDAC in liver cancer progression or metastases. However, HDAC expression has been reported to be altered in liver cancer tissues (Quint et al., 2011; Wu et al., 2010). The available data on the role of HDACs in cancer indicated that there is more than one mechanism by which HDACs function in cancer development. For example, HDACs usually function via forming a co-repressor complex such as transcriptional co-repressors mSin3, N-CoR, and SMRT (Wong and Privalsky, 1998; Karagianni and Wong, 2007). Other studies indicated that HDACs could regulate the expression of a large number of genes by direct interaction with transcription factors such as E2f, Stat3, p53, the retinoblastoma protein, NF-κB, TFIIE, etc (Lin et al., 2006).

In order to understand the molecular events regulated by HDACs in certain pathological conditions such as cancer, it is necessary to identify and characterize the protein complexes involved in that particular cell type as the composition of various HDAC complexes are found to be cell type dependent (Delcuve et al., 2013). Scientists have applied various methods to identify protein-protein interactions. Initially, large-scale protein interaction studies were performed with the yeast two hybrid technology (Uetz et al., 2000, Walhout et al., 2000, Ito et al., 2001) and more recently, affinity purification coupled with mass spectrometry (AP-MS) has become the method of choice for the analysis of protein complexes under near-physiological conditions (Gingras et al., 2007, Kocher and Superti-Furga, 2007).

Among class 1 HDAC family members, the expression of HDAC1 was reported to be altered in liver cancers and hdac1 also regulate zebrafish liver formation (Farooq et al., 2008; Wu et al., 2010; Yang et al., 2010; Quint et al., 2011; Lachenmayer et al., 2012). This suggests a critical role of HDAC 1 in liver formation and liver carcinogenesis. It could be possible that HDAC1 interact with other proteins possibly making protein complexes in order to function in liver carcinogenesis. The existence of HDAC1 protein complex specifically in liver cancer is not known, so we have tried to investigate, if there is any protein complex associated with HDAC1in liver cancer.

Initially, large-scale protein interaction studies were performed with the yeast two hybrid technology (Uetz et al., 2000; Walhout et al., 2000; Ito et al., 2001). More recently, affinity purification coupled with mass spectrometry (AP-MS) has become the method of choice for the analysis of protein complexes under near-physiological conditions (Gingras et al., 2007; Kocher and Superti-Furga, 2007). We have adopted double affinity purification (SH-tag) coupled with direct LC-MS/MS to fish out the HDAC1 interacting proteins in liver cancer cells. Direct LC-MS/MS has poorly been exploited in previous high throughput AP-MS studies but can significantly enhance sensitivity, as the entire affinity-purified sample can be analyzed by a reversed-phase liquid chromatography unit coupled to a mass spectrometer in a one single step. The major bottleneck for efficient direct LC-MS/MS analysis is the complexity of sample and impurities. We overcome these two problems adopting the double affinity purification and in addition, the second purification step efficiently removes detergent, protease inhibitors and eluting reagents (e.g. biotin) present in the sample, which would interfere with subsequent LC-MS/MS. In order to overcome the problem of expression of bait protein by the mammalian cells, we have successfully adopted the recombinational cloning strategy and expression constructs were generated using

human orfeome libraries with homologous recombination by Flp recombinase. The presence of an Flp recombination target site (FRT) in the resulting expression constructs supported the rapid generation of bait expressing cell lines by Flp-mediated recombination. To improve the yield, a novel double-affinity purification protocol was adopted. Protein complexes were isolated through a small double-affinity tag (SH-tag) consisting of a streptavidin-binding peptide and a hemagglutinin (HA) epitope tag. Following induction of isogenic bait expression using tetracycline, SH-tagged and associated proteins were first bound to an affinity column containing a modified version of streptavidin (Junttila et al., 2005) and specifically eluted with biotin onto an anti-HA antibody column. To further reduce the chances of purifying contaminanted proteins, the signal intensity ratio from the mock transfected cells were used for background subtraction. This modified protocol yielded high percentage of reproducibility and the variations of the biological replicates A, B and C were below 10% (Figure) and showed the highest reproducibility in any of the AP-MS experiments reported so far.

We have identified several subunits of T complex proteins (TCP1) a cellular chaperones as binding partners for HDAC1 in liver cancer cells. The chaperonin-containing t-complex polypeptide 1 (CCT) is a hetero-oligomeric molecular chaperone that assists in the folding of actin, tubulin and other cytosolic proteins. TCP1 proteins are also implicated in various cancers as different subunits have been shown to be expressed in cancer cells and cancer tissues from patients as well (Myung et al., 2004). The CCTα and CCTβ proteins were shown to be significantly higher in tumor tissues as compared to non tumor tissues in patients with hepatocellular carcinoma (Yokota et al., 2001). T-complex protein 1 ζ have been suggested as novel indicators for evaluating lymph node metastasis in colorectal cancer (Yue et al., 2009). Inhibition of cytosolic chaperonin CCTζ-1 expression depletes proliferation of colorectal carcinoma in vitro (Qian-Lin et al., 2010). All these studies just demonstrated expression of chaperonin protein in various cancers or cancer cell lines but none of them has analyzed the regulation of these proteins. Molecular chaperones are key players controlling the biogenesis of macromolecular assemblies, and also implicated in HDAC protein complex assembly. HDAC 3 forms a protein complex with nuclear hormone receptor co-repressor SMRT or N-CoR, well known as HDAC3- SMRT complex (Wen et al., 2000; Guenther et al., 2001). Interestingly, TCP-1 was reported as integral component for this assembly and TCP-1 ring is required for the assembly of the SMRT– HDAC3 repression complexes (Guenther et al., 2002). The presence of similar requirement of TCP1 for HDAC1 complexes in not known and this could be the first clue to further explore physical association of TCP1 and HDAC1. Another protein family which showed very high affinity with HDAC1 in HepG2 cells was prefoldin (PFDN). Actually, PFDN5 having the highest affinity with HDAC1 among all the identified interactors. Prefoldin is a heterohexameric chaperone protein which has the ability to capture unfolded actin. So far five prefoldin polypeptides (prefoldin 1-5), have been identified. Prefoldin 1 is a 122 amino acid protein that binds specifically to cytosolic chaperonin (c-cpn) and transfers target proteins to it. Prefoldin 3 (VBP1 or VHL binding protein-1) forms complexes with VHL and is translocated from perinuclear granules to the nucleus or cytoplasm. Prefoldin 4 is a possible transcription factor. Prefoldin 5 (c-Myc-binding protein Mm-1, Myc modulator 1 or MM-1) is a c-Myc binding protein (Tsuchiya et al., 1996; Brinke et al., 1997; Mori et al., 1998; Vainberg et al., 1998; Fujioka et al., 2001; Satou et al., 2001).

We reported here for the first time, various subunits of TCP1 and PFDN as potential interacting proteins with HDAC1 in HepG2 cells. This novel finding could lead to design effective therapeutics targeting HDAC1 as an effective therapy to treat hepatocellular carcinoma.

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