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TOPIC HIGHLIGHT

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Alcohol-induced alterations of the hepatocyte cytoskeleton

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Abstract

The hepatocyte cytoskeleton consists of three filamentous networks: microtubules, actin microfilaments and keratin intermediate filaments. Because of the abundance of the proteins that comprise each system and the central role each network plays in a variety of cellular processes, the three filament systems have been the focus of a host of studies aimed at understanding the progression of alcohol-induced liver injury. In this review, we will briefly discuss the hepatic organization of each cytoskeletal network and highlight some components of each system. We will also describe what is known about ethanol-induced changes in the dynamics and distributions of each cytoskeletal system and discuss what is known about changes in protein expression levels and post-translational modifications. Finally, we will describe the possible consequences of these cytoskeletal alterations on hepatocyte function and how they might contribute to the progression of liver disease.

Key words: Ethanol; Hepatotoxicity; Tubulin; Actin; Keratin

INTRODUCTION

The hepatocyte cytoskeleton consists of three major filamentous networks: microtubules, actin and cytokeratins. Recent genomics and proteomics studies have revealed that components of each of these networks are altered in ethanol-treated hepatocytes and/or liver. For example, the expression levels of many different genes encoding for cytoskeletal proteins are changed in ethanol-treated systems (Table 1). Similarly, cytoskeletal protein expression levels are altered by ethanol exposure (Table 2). However, it is important to note that changes in gene expression do not necessarily correlate with changes in protein expression. Furthermore, ethanol metabolites have been shown to form adducts with various proteins of the cytoskeleton (Table 3). More recently, it has become apparent that many post translational modifications of the natural repertoire are also induced by ethanol exposure (Table 3). These alcohol-induced alterations strongly suggest that cytoskeleton structure and function is impaired in ethanol-treated hepatocytes. Because the cytoskeleton is vital to innumerable cellular processes, these alterations likely have profound effects on proper hepatocyte function and lead to the progression of alcoholic liver injury. In this review, we will describe what is

known about specific alterations of components of the three cytoskeletal networks and discuss how they may contribute to ethanol-induced hepatotoxicity.

TUBULIN

Microtubules are made of repeating units of α- and β-tubulin heterodimers that form protofilaments, which in turn assemble into hollow tubes consisting of 13 protofilaments arranged in parallel. Microtubules exist as both dynamic and stable polymers. The latter population is characterized by a longer half-life, resistance to microtubule poisons (e.g. cold and nocodazole) and by specific post-translational modifications on the α-tubulin subunit[22]. These modifications include the removal of a carboxy-terminal tyrosine, polyglutamylation, polyglycylation and acetylation of lysine 40[22]. In hepatocytes, cell surface polarity is reflected in the asymmetric organization of microtubules. Unlike in non-polarized cells where microtubules emanate from a juxta-nuclear microtubule organizing center, in polarized cells, there is accumulating evidence that microtubules are instead (or additionally?) organized from sites at or near the apical plasma membrane (Figure 1). The emanating microtubules are oriented with their minus ends at the apical surface and their plus ends attached to or near the basolateral membrane[23]. Because microtubules are central to multiple cellular processes including organelle placement, mitosis and vesicle motility *via* motor proteins, they have been the subject of a host of studies examining the effects of chronic ethanol exposure on hepatic function.

Acetaldehyde adduction

As ethanol is metabolized, acetaldehyde is produced. This highly reactive metabolite can readily covalently modify proteins, DNA and lipids[21,24-33]. Many proteins have been shown to be modified by acetaldehyde including tubulin, actin, calmodulin, hemoglobin, hepatic enzymes and plasma proteins[21,29-31,33,34]. In general, acetaldehyde is thought to form stable adducts with the ε-amino group of lysine residues[32,35,36]. The hypothesis is that these cumulative covalent modifications disrupt the normal functioning of hepatic proteins leading to cell injury.

One of the best-studied target proteins for acetaldehyde is α-tubulin[32]. *In vitro*, soluble tubulin dimers purified from either bovine brain[18,19] or rat liver[20] were found to be much more highly adducted than preformed microtubules (Table 3). Adduction occurred preferentially on the α-tubulin subunit at a highly reactive lysine[18,19]. Further examination revealed that adduction of this highly reactive lysine on α-tubulin drastically impaired *in vitro* microtubule polymerization[18,19]. Assays using low acetaldehyde: tubulin dimer levels further revealed that impaired microtubule formation occurred at substoichiometric amounts of acetaldehyde (0.2 mol acetaldehyde/mol tubulin) suggesting that small levels of adduction can have far reaching effects on microtubule function[37]. More recently it was shown that tubulin purified from ethanol-fed rat livers displayed impaired polymerization relative to control[7]. Although consistent with an acetaldehyde-induced impairment, the presence of adducts on the purified tubulin was not confirmed.

Impaired microtubule polymerization has also been examined in isolated hepatocytes from alcohol-fed rats[7]. After removing nocodazole (a reversible microtubule

Table 1 Ethanol-induced changes in cytoskeleton-associated protein gene expression

Gene product	Expression levels	System	EtOH exposure	Ref.
α1-tubulin	Increased	Rat liver	Chronic (IG)	[1]
β5-tubulin	Increased	Rat liver	Chronic (IG)	[1]
β-tubulin	Decreased	Rat liver	Chronic	[2]
Kinesin 2c	Increased	Mouse liver	Acute	[3]
Tau	Decreased	Rat liver	Chronic	[2]
αE integrin	Increased	Rat liver	Chronic (IG)	[4]
PDZ and LIM domain protein 1	Decreased	Rat liver	Chronic	[5]
Thymosin β-like protein	Decreased	Rat liver	Chronic	[2]
Myosin 1b	Decreased	HepG2 (+ CYP2E1)	Acute	[4]
Myosin 1E	Decreased	Rat liver	Chronic	[2]
Myosin light chain 2	Decreased	Rat liver	Chronic	[2]
Myosin light chain 3	Decreased	Rat liver	Chronic	[2]
Titin	Decreased	HepG2 (+ CYP2E1)	Acute	[4]
Myosin VII A and Rab interacting protein 1	Increased	Rat liver	Chronic (IG)	[6]
ZO-2	Decreased	Rat liver	Chronic	[5]
Cadherin 17	Increased	Rat liver	Chronic (IG)	[4]

IG: Intragastric.

Table 2 Ethanol-induced changes in cytoskeletal protein expression levels

Protein	Expression levels	System	EtOH exposure	Ref.
α-tubulin	No change	WIF-B cells, rat liver, isolated hepatocytes	Chronic	[7,8]
α-tubulin	Decreased	Rat liver	Chronic	[9]
Dynein	No change	Rat liver	Chronic	[7]
Kinesin	No change	Rat liver	Chronic	[7]
Actin	No change	WIF-B cells, rat liver	Chronic	[10,11]
Cortactin	No change	WIF-B cells, Rat liver	Chronic	[11]
Vinculin	No change	Rat liver	Chronic	[10]
FAK	No change	Rat liver	Chronic	[10]
Paxillin	No change	Rat liver	Chronic	[10]
RhoA	No change	Rat liver	Chronic	[12]
Rac	Increased	Rat liver	Chronic	[12]
Cdc42	Increased	Rat liver	Chronic	[12]
α1 integrin	Increased	Rat liver	Chronic	[10,13]
α5 integrin	Increased	Rat liver	Chronic	[10,13]
β1 integrin	Increased	Rat liver	Chronic	[10,13]
Keratin 8[1]	No change	Rat liver	Chronic	[14]
Keratin 18[1]	No change	Rat liver	Chronic	[14]

[1]Please see the text for a description of changes in keratins 8 and 18 protein expression levels that are associated with Mallory-Denk bodies in ethanol-fed human and mouse hepatocytes.

Table 3 Ethanol-induced cytoskeletal protein modifications

Modification	Protein	System	EtOH exposure	Ref.
Lysine acetylation	α-tubulin	WIF-B cells, rat liver	Chronic	[8]
	β-actin	Rat liver	Chronic	[11]
	Cortactin	Rat liver	Chronic	[11]
Dephosphorylation	Keratin 8[1]	Rat liver	Chronic	[15]
	Keratin 18[1]	Rat liver	Chronic	[15]
Phosphorylation	Keratin 8	Rat liver	Acute	[16]
	Keratin 18	Rat liver	Acute	[16]
Cysteine oxidation	50 kDa dynactin subunit	Mouse liver	Chronic	[17]
	α-actin	Mouse liver	Chronic	[17]
	β-actin	Mouse liver	Chronic	[17]
	Keratin 1	Mouse liver	Chronic	[17]
	Keratin 2	Mouse liver	Chronic	[17]
	Keratin 9	Mouse liver	Chronic	[17]
	Keratin 10	Mouse liver	Chronic	[17]
	Keratin 14	Mouse liver	Chronic	[17]
	Keratin 16	Mouse liver	Chronic	[17]
Lysine adduction by acetaldehyde	α-tubulin	*In vitro*[2]	N/A	[18-20]
	MAPS[3]	*In vitro*[4]	N/A	[18]
	Actin	*In vitro*[5]	N/A	[21]

[1]Please see the text for a description of the multiple post-translational modifications on keratins 8 and 18 that are associated with Mallory-Denk bodies in ethanol-fed human and mouse hepatocytes; [2]Purified from bovine brain or rat liver; [3]Microtubule associated protein and motor fraction; [4]Purified from bovine brain; [5]Purified from rabbit skeletal muscle.

depolymerizing agent), microtubule regrowth was monitored morphologically and was found to be significantly impaired in ethanol-treated hepatocytes. Ethanol-treated WIF-B cells exhibited a similar tubulin phenotype where microtubule regrowth after nocodazole washout was impaired[8]. Although the formation of tubulin-adducts has not been defined *in vivo*, these results are consistent with the effects of acetaldehyde on tubulin assembly *in vitro*.

Acetaldehyde has also been shown to form adducts on a purified fraction of microtubule associated proteins (MAPs) and motors at levels 1.5-fold more than tubulin[18] (Table 3). Although the consequences of these modifications have not been explored, vesicle motility in isolated hepatocytes from ethanol-fed rats was found to be significantly decreased suggesting alcohol-induced motor dysfunction[38]. Although the microtubule activated ATPase activities of kinesin or dynein purified from ethanol-exposed livers was not altered[38], this does not exclude the possibility that their microtubule binding properties are altered *in vivo* thereby leading to decreased motility. This is consistent with our recent findings that histone deacetylase 6 (HDAC6) binding to endogenous microtubules was impaired in ethanol-treated WIF-B cells and that this impairment partially required ethanol metabolism[39]. Measuring HDAC6 tubulin deacetylase activity further revealed that ethanol did not impair HDAC6's ability to bind or deacetylate exogenous tubulin suggesting that tubulin from ethanol-treated cells was modified (acetaldehyde adducted?) thereby preventing HDAC6 binding[39]. Similarly, tubulin modifications may prevent motor or MAP binding leading to impaired vesicle motility or altered microtubule dynamics.

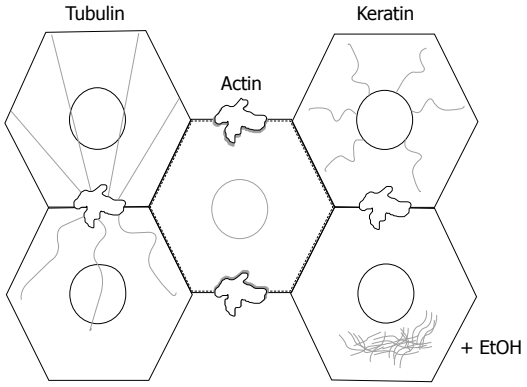

Figure 1 Ethanol alters cytoskeletal organization in hepatocytes. In control cells (top left cell), microtubules emanate from sites at or near the apical plasma membrane with their plus ends attached to or near the basolateral plasma membrane. In the presence of ethanol (bottom left cell), microtubules appear thicker, shorter and more gnarled. In contrast, ethanol does not alter actin filament organization (middle cell). Both control and ethanol-exposed actin filaments form a dense sub-cortical web at both the apical and basolateral plasma membranes. Keratin filaments normally form dense cortical networks originating from the apical and basolateral plasma membrane in hepatocytes (top right cell). In the presence of ethanol, keratin filaments accumulate in dense cytoplasmic inclusions known as Mallory-Denk Bodies (bottom right cell).

Acetylation

Although tubulin polymerization was impaired in ethanol-treated WIF-B cells, when microtubules were examined morphologically, they resembled the so-called "stable" polymers (Figure 1). Antibodies to acetylated lysine 40 on α-tubulin confirmed their identity morphologically and revealed biochemically that ethanol-treated cells had approximately three-fold more acetylated α-tubulin than control cells. Consistent with increased acetylated α-tubulin levels, microtubules in ethanol-treated WIF-B cells were more stable. We further confirmed these results in livers from ethanol-fed rats indicating the findings have physiologic importance[8]. Because microtubule hyperacetylation and stability increased with increased time of ethanol exposure or concentration, and was prevented by 4-methylpyrazole and potentiated by cyanamide, we conclude that increased acetylation requires alcohol metabolism and is likely mediated by acetaldehyde[8]. Thus, ethanol metabolism impairs tubulin polymerization, but once microtubules are formed they are hyperstabilized.

Possible consequences of tubulin modifications

Because microtubules are central to multiple cellular processes, changes in their dynamics will likely alter hepatic function. An active area of research has been aimed at understanding the relationship between protein trafficking and alterations in microtubule dynamics. Not only is protein trafficking microtubule-dependent, the trafficking of many hepatic proteins is also impaired by ethanol[40-43]. Thus, the observed alcohol-induced defects in protein trafficking may be explained by changes

in microtubule dynamics, altered tubulin/MAP/motor post-translational modifications or impaired interactions between microtubules and other proteins. Although the precise relationship between acetaldehyde-modified tubulin and defects in protein trafficking is not known, a link between tubulin acetylation and impaired protein trafficking is emerging. Of particular interest are studies performed in WIF-B cells that used a novel microtubule depolymerizing drug, 201-F[44]. Poüs *et al*[44] showed that this drug specifically depolymerized deacetylated microtubules; only acetylated populations remained. This implies that 201-F is a specific poison for dynamic microtubules. They further examined specific protein transport steps and found that secretion and transcytosis are dependent on dynamic (deacetylated) microtubules while delivery of basolateral glycoproteins to the sinusoidal surface is dependent on stable (acetylated) microtubules. Can the defects in protein trafficking in ethanol-treated cells be explained by increased acetylation and increased stability of microtubules?

To test this possibility, we compared the trafficking of selected proteins in control cells and cells treated with ethanol or the HDAC6 inhibitor, trichostatin A (TSA). Importantly, TSA led to increased microtubule acetylation and stability to the same extent as ethanol[45]. Both treatments led to the impaired clathrin internalization of asialoglycoprotein receptor and aminopeptidase N[45]. Interestingly, the internalization of 5'nucleotidase a GPI-anchored protein, was not impaired indicating the effect was selective (i.e. only certain internalization mechanisms were impaired) and specific (i.e. the changes were due to altered microtubule dynamics)[45]. Furthermore, we determined that albumin secretion was impaired in TSA-treated cells. Thus, increased microtubule acetylation and stability, in part, can explain ethanol-induced defects in protein trafficking. It remains to be determined the specific mechanism by which tubulin acetylation impairs protein trafficking and whether tubulin acetaldehyde adducts also contribute to the impairments observed.

ACTIN

Actin is a ubiquitous cytoskeletal protein that exists as both a monomer (G-actin) and a filamentous polymer (F-actin). These dynamic filaments are the primary component of many cellular structures including stress fibers in adherent cells, the contractile ring in dividing cells and lamellipodia at the leading edge of migrating cells. The actin cytoskeleton has a unique organization in many polarized cells. In general, actin microfilaments extend to the basolateral membrane and form attachments through interactions with proteins of zonulae adherens, tight junctions and focal adhesions. At the apical surface, actin is found as the core filament of microvilli and also as a dense sub-cortical web[46-48] (Figure 1). At the basolateral domain, the actin-associated proteins, fodrin and ankyrin, form a scaffold that restricts the movements of certain integral membrane proteins, thereby stabilizing the basolateral protein population[46] (Figure 1). Actin microfilaments are involved in a host of cellular processes ranging from cytokinesis to establishment of epithelial cell polarity. Interestingly, actin is also known to be important in regulating clathrin vesicle fission, vesicle trafficking *via* myosin motor proteins and cell attachment and spreading - important processes known to be impaired by ethanol exposure. Thus, the effects of ethanol metabolism on the actin cytoskeleton deserve close examination.

Acetaldehyde adduction

Like tubulin, actin contains several reactive lysines making it an attractive candidate for acetaldehyde-adduct formation. When the covalent binding of radiolabeled acetaldehyde to purified actin was examined, stable adducts were formed under both reducing and non-reducing conditions[21] (Table 3). Interestingly, globular (G) actin formed considerably more adducts with acetaldehyde than filamentous (F) actin, and the monomer efficiently competed for acetaldehyde adduction when co-incubated with albumin, another known adducted protein. However, despite the readily adducted G actin, actin polymerization was not impaired[21]. This does not rule out the possibility that adduction can lead to altered actin dynamics *in vivo* or impair binding of actin to its binding partners or myosin motors that may explain some of the observed defects in cell spreading and protein trafficking described below.

Hepatocyte attachment and spreading

Although actin adduction does not alter actin polymerization, ethanol has been shown to impair the function of actin-regulated processes. Among these are the observed defects in hepatocyte-extracellular matrix (ECM) attachment and subsequent cell spreading. Initially, hepatocyte attachment occurs when the transmembrane adhesion proteins, integrins, bind to ECM components including fibronectin, collagen, and laminin. Integrin clustering leads to the formation of focal adhesions and induces actin reorganization *via* the activation of Rho family GTPases. Focal adhesion turnover and actin dissociation from integrins are required for subsequent cell spreading. In isolated hepatocytes from ethanol-fed livers, a significant increase in the expression of α_1, α_5 and β_1 integrins was observed[10,13] (Table 2). Despite the increased levels of these ECM receptors, decreased hepatocyte attachment to their ECM ligands was observed and subsequent cell spreading was significantly impaired[13,49,50]. These somewhat disparate findings suggest that integrin overexpression was compensating for decreased ECM attachment. Furthermore, this defect was found to be more prominent in the perivenous hepatocytes, sites where alcoholic liver injury predominates[49].

Because actin cytoskeleton rearrangement is required for cell ECM attachment and subsequent cell spreading, a more recent study examined the downstream Rho GTPases in isolated hepatocytes from ethanol-fed rats.

This family of small molecular weight GTPases regulates actin rearrangement, including the formation of lamellipodia (*via* Rac), filopodia (*via* Cdc42) and stress fibers (*via* RhoA). Like for integrin expression levels, both Rac and Cdc42 levels were increased in isolated hepatocytes from ethanol-fed rats suggesting another possible compensatory mechanism to regain proper adhesion[12] (Table 1). However, despite the increased total protein levels of these two GTPases, the activated GTP-bound forms of Rac and Cdc42 were significantly decreased in the presence of ethanol[12]. Because no change in GTPγS binding to either GTPase was observed, the authors suggest that GTP/GDP exchange by either the guanine nucleotide exchange factors or activating proteins is impaired, not GTP binding itself. Furthermore, when activation profiles of Rac and Cdc42 were examined during cell spreading, decreased Rac activation was observed only for the first 24 h. In contrast, decreased activation of Cdc42 persisted[12]. This might be due, in part, to the additional role that Cdc42 plays in the establishment of cell polarity such that its activity is required longer. In contrast, RhoA activation or protein expression levels were not changed by ethanol exposure at steady state or during cell spreading assays. This might be explained by the absence of stress fibers in hepatocytes. Together, these studies suggest that decreased hepatocyte ECM attachment and spreading is due to altered Rac and Cdc42 GTPase activity that results in altered actin reorganization such that cell spreading is impaired.

Other actin cytoskeleton modifications

More recently, ethanol has been shown to induce additional protein modifications besides the well characterized acetaldehyde adduction. For example, a recent study examined the widespread CYP2E1-mediated cysteine oxidation in ethanol-fed mouse livers. Over 90 cytosolic proteins were found to be cysteine-oxidized including α and β actin and a dynactin subunit (Table 3). Although the functional consequences of this modification are not currently known[17], it is attractive to postulate that the modified cysteine residues lead to altered actin dynamics leading to impaired hepatocyte function.

Our recent proteomics studies have identified β-actin as one of 40 other non-nuclear rat liver proteins that is hyperacetylated upon ethanol-exposure[11,51]. We confirmed its ethanol-induced hyperacetylation and further determined that cortactin, a known actin binding protein, was also hyperacetylated in these samples[11] (Table 3). Although the acetylated lysine(s) has not been identified, the possible functional consequences of this modification may be gleaned from other studies. Previous work from our lab and others has found that ethanol impairs clathrin-mediated endocytosis, secretion and delivery of newly synthesized membrane proteins to the basolateral membrane[40-43]. In addition, studies using TSA (a histone deacetylase inhibitor), have linked these impairments to increased protein acetylation[45]. Since both actin and its binding partner, cortactin, are likely required for clathrin-vesicle formation at the plasma membrane and TGN[52,53], an intriguing possibility is that actin hyperacetylation may contribute to the observed alcohol-induced defects in protein trafficking. In general, cortactin is thought to promote actin polymerization at sites of vesicle formation and recruit dynamin (a GTPase required for vesicle fission) to the necks of budding vesicles[52,53]. At present, the exact mechanism by which cortactin, actin and dynamin function to promote vesicle release is not yet completely elucidated. However, acetylation of cortactin is known to prevent its association with actin and alters its subcellular localization[54]. From these results, we propose that alcohol-induced hyperacetylation leads to decreased interactions between actin and cortactin such that cortactin is no longer recruited to sites of clathrin-vesicle formation thereby inhibiting associations with dynamin and subsequent vesicle fission. We are currently testing this exciting possibility.

In a recent system-wide survey for lysine acetylation, approximately 200 proteins were found to be acetylated including many cytoskeletal proteins[55]. In addition to γ-actin, this list included many known actin-binding proteins, suggesting a possible general regulatory role for this modification on actin function. For example, profilin (involved in microfilament elongation) and cofilin and thymosin (involved in actin filament destabilization) were among those identified, suggesting acetylation may regulate actin dynamics. The screen also detected moesin, an ERM family member, required for actin assembly at the apical plasma membrane, suggesting that lysine acetylation may also regulate the formation of the cortical actin web in hepatocytes. However, it is not yet known whether ethanol induces hyperacetylation of these or other actin binding proteins. Nonetheless, we predict that many actin-dependent processes are regulated by lysine acetylation and that ethanol exposure leads to increased hyperacetylation thereby provoking altered regulation. Clearly, these exciting hypotheses need to be rigorously tested.

KERATIN

In general, the cytokeratin intermediate filament system is composed of polymerized dimers consisting of one acidic (Type Ⅰ) and one basic (Type Ⅱ) keratin subunit. Although over 50 keratin isoforms have been identified, hepatocytes express only keratin 8 (Type Ⅱ) and keratin 18 (Type Ⅰ). The developing embryonic liver also expresses keratin 19 (Type Ⅰ), and some very low levels of this isoform may be also expressed in adult hepatocytes[56]. In most polarized epithelial cells, keratin filaments form dense apical cortical networks[57]. In contrast, apical and basolateral cortical keratin filaments are observed in hepatocytes[57,58] (Figure 1). The polarized distribution of the keratin filaments have led some researchers to suggest that they play an important role in the establishment and maintenance of epithelial cell polarity and in polarized secretion[57]. Unlike for microtubules and actin, there are no specific motor proteins

associated with intermediate filament systems suggesting this filament system does not support vesicle motility directly. However, several intermediate filament associated proteins (IFAPs) have also been identified that are thought to modulate filament assembly and associations with actin filaments and microtubules[59]. These associations are required for the polarized distribution of both actin and microtubules suggesting the role of keratins in regulating polarity and polarized protein trafficking is likely indirect by providing a polarized scaffold for filament orientation[57].

Keratin post-translational modifications

For almost 100 years, Mallory-Denk bodies have been recognized as a pathological marker in patients with alcoholic liver disease. Because there have been a number of excellent recent reviews[56,59-62] written about the composition and formation of these inclusions, we will only discuss them briefly here. Morphologically, Mallory-Denk bodies are dense cytoplasmic inclusions formed of fibrillar keratin, chaperones, components of the protein degradation machinery and other proteins[56,59-62] (Figure 1). Interestingly, the keratin filaments in these inclusions are highly post-translationally modified. They are hyperphosphorylated, transamidated (*via* transglutaminase 2), ubiquitinated and partially degraded[59,62]. In the alcoholic, Mallory-Denk bodies are thought to form in response to oxidative stress that triggers many interrelated cellular responses including the upregulation of keratin expression (keratin 8 > keratin 18) and its subsequent post-translational modification[60,62]. Other hepatic proteins are misfolded in response to oxidative stress and associate with chaperones. Damaged proteins are also ubiquitinated and targeted for degradation *via* the proteasome or autophagosomes. The accumulated proteins overwhelm the degradative machinery that is already compromised by ethanol treatment[63] resulting in the formation of dense inclusions. Whether Mallory-Denk bodies contribute to the progression of alcoholic liver disease, are inert or are hepatoprotective is currently not clear[59,62].

Another open question is the extent to which keratin intermediate filament function is impaired before the appearance of Mallory-Denk bodies. In general, Mallory-Denk bodies are formed at the expense of an intact keratin filament system. Do keratins present in the intact filament system display lower levels of the various post-translational modifications before inclusion formation? Does this alter keratin function? A few studies performed in rat hepatocytes suggest this is the case. In isolated hepatocytes after acute ethanol exposure both keratins 8 and 18 were found to be hyperphosphorylated[16] (Table 3). In ethanol-fed rats, the keratin network was also found to be disrupted with increased cytosolic staining observed[58]. One attractive hypothesis is that keratin phosphorylation leads to altered associations with IFAPs thereby leading to altered keratin function. However, it is important to point out that rats do not form Mallory-Denk bodies, so whether these changes occur in humans will be important to determine. It is also not known whether keratins 8 or 18 or other hepatic IFAPs are targets for adduction by ethanol metabolites. It is interesting to point out that a number of non-hepatocyte keratin isoforms were found to be cysteine-oxidized in chronically-fed mouse livers (Table 3) suggesting the intermediate filament networks in other cell types may be altered upon ethanol exposure. Clearly, this hypothesis merits further attention.

CONCLUSION

In this review, we have briefly discussed the hepatocyte cytoskeleton and the known ethanol-induced impairments in its structure and function. In general, elements of the actin, microtubule and keratin filament networks undergo changes in distributions, expression levels or post-translational modifications upon exposure to ethanol. While the direct effects of these alterations are still under investigation, it is attractive to speculate that they lead to profound changes in hepatic function. Continued research in this field will not only increase our understanding of the pathogenesis of alcoholic liver disease, but may also provide novel therapeutic approaches to treatment.

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