International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 1504-1507

Received: 10-01-2019
Accepted: 13-02-2019

Sangamitra RKR
M.V.Sc Scholar, Department of Veterinary Physiology, Madras Veterinary College, Chennai, Tamil Nadu, India

G Suganya
Assistant Professor, Department of Veterinary Physiology, Madras Veterinary College, Chennai, Tamil Nadu, India

V Leela
Professor and Head, Department of Veterinary Physiology, Madras Veterinary College, Chennai, Tamil Nadu, India

M Balagangatharathilagar
Assistant Professor, Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai, Tamil Nadu, India

Correspondence
Sangamitra RKR
M.V.Sc Scholar, Department of Veterinary Physiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Cytokeratin 18: Skeletal protein of hepatocytes, its functions and diagnostic significance

Sangamitra RKR, G Suganya, V Leela and M Balagangatharathilagar

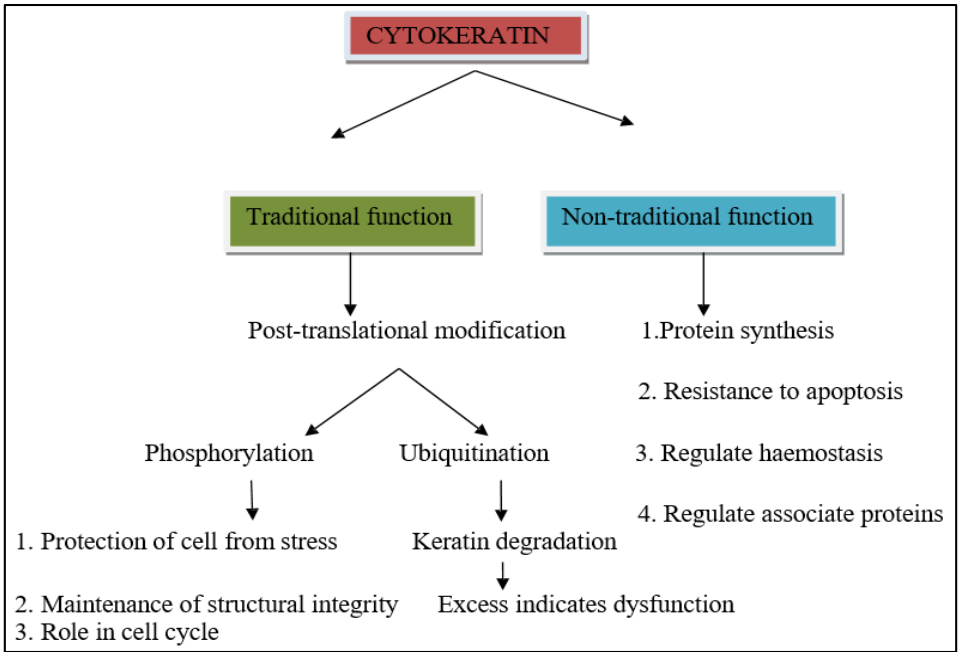
Abstract
Liver, a complex organ performs various functions to maintain homeostasis in the body. The regenerative capacity of the liver is enormous and any injury to the liver can be detected only when 60-70 percent of the organ has been damaged. Injury to any organ can be detected by the use of biomarkers. Biomarkers are any biological molecule which acts as measurable indicators of biological process and one such biomarker for hepatic disease is Cytokeratin 18, an intermediate filament protein predominantly expressed in hepatocytes along with cytokeratin 8. Apart from maintaining the mechanical stability to cell structure, cytokeratin18 unveils highly dynamic features such as cell to cell signaling, progression of cell cycle, resistance to apoptosis, regulation of protein synthesis and hemostasis. This review explores the various studies related to functions mediated by cytokeratin 18 and its role as a potential biomarker for different pathological alterations in accordance with hepatic diseases.

Keywords: Hepatocyte, cytokeratin 18, dynamic functions, apoptosis, hepatic biomarker

1. Introduction
Cytoskeletal proteins are a framework of proteinaceous structural filaments that are present within the cellular cytoplasm to maintain the structure of a cell. Based on the filament diameter, they are classified into three major cytoskeletal filamentous proteins as Microfilaments (6nm), made up of actin, Microtubules (25nm) which are hollow cylinders and Intermediate filaments (10nm) heteropolymers [10]. Intermediate filament proteins helps in organizing the internal 3- dimentional cellular structure by holding the cell organelles in position in the cytoplasm. Cytokeratin 18 is a type I acidic keratin intermediate filament protein which are expressed in tissue specific manner in the cytoplasm of simple glandular epithelial cells such as liver, gut and pancreas. In hepatocyte, it always pairs with Keratin 8, a type II basic intermediate filament through coil - coil interaction to perform its function such as cell structural integrity, protein synthesis [6, 24].

2. Structure
The gene for keratin 18 expression is K18 gene which is 3791 base pairs in length and is encoded by seven exons. The K18 gene contains an unusual slice site AG/GC at intron 3 instead of AG/GT sequence which is present in chromosome 18. The K18 is typical as it has 7 exons instead of 8 where the last exon codes for the tail domain [19]. The intermediate filament of hepatocyte is formed by cytokeratin 8 and 18, each consisting of a centrally placed α-helical rod domain, made of 310 - 350 aminoacids, has subdomains 1A, 1B, 2A, 2B which are connected to each other by L1, L12 and L2 linkers. The central rod domain is guarded on both sides by a non α-helical N terminal (head) and C terminal (tail) domains, both have subdomains V1, H1 and H2, V2 [10]. VEVD238 and DALD397 are the caspase cleavage sites. VEVD238 is found in the central rod domain and DALD397 site is found only in the tail domain of cytokeratin 18 [2].

3. Functions of cytokeratin 18



3.1 Post translational modification

Post translational modification is an important step in keratin intermediate filaments in order to perform filament disassembly and reorganization. The two important post translational modification noticed in keratin filaments are phosphorylation and glycosylation [4]. Colcemid / nocodazole treatment shows drastic increase in the phosphorylation of cytokeratin 18, predominantly within the NH2 terminal of 125 aminoacids which are included in the proximal and the head rod domains especially at the serine residues. Mitotic arrest is also mediated via glycosylation [3]. Ser-52 in K18 is the major site of phosphorylation during interphase of cell cycle. When the cells are treated with okadaic acid, they show alterations in filament due to mutation of Ser-52 site. Those cells with K18 Ser---ala mutations were introduced into insectcells and were compared with PO4- labeled tryptic peptides after labelling with normal colonic tissues and studies with K18 Ser---ala mutant mammalian cells revealed absence / minimal phosphorylation at the site when compared with the wild type K18 [18]. The structural integrity during mechanical stress is maintained by Cytokeratin 8/18 filaments in studies done in homozygous (HMZ), Heterozygous and Wild-type mutant mice models [21].

3.2 Role in cell cycle

During S/G2/M phases of cell cycle, phosphorylation of K8/18 is a significant step for the association of 14-3-3 proteins inorder to protect the cells from the consequence of heat stress or okadoic acid treatment. In invitro study, has demonstrated 14-3-3 proteins as a solubility factor in cells during S/G2/M phases of cell cycle by interacting with the keratin 18 after its phosphorylation by various signal transduction kinases [12]. The 14-3-3 proteins forms phosphoamino acid binding through typical groove formation with the lysine and arginine residues. By invitro labelling and 2D gel analysis, it was identified that the Keratin 18 phosphorylation was more pronounced than keratin 8, even though basal levels of keratin 18 phosphorylation is less than keratin 8. With the treatment of cytosolic, NP40 and Emo soluble they noticed the increase in the solubility component which results in the reduction of intracellular signalling till mitosis. At the end of mitosis, treatment 14-3-3 protein complex with phosphatases resulted in the dephosphorylation of keratin 18 from 14-3-3 protein which then continues with reorganisation of keratin filament for the normal cell cycle progression. They putforth the idea of various roles of keratin as chaperones, linkers for 14-3-3 protein function in cell signaling [20].

3.3 Resistence to apoptosis

To maintain tissue homeostasis, special mechanism called apoptosis is mediated by the body to destruct the abnormal or damaged cells [28]. *In vitro* studies using various cell lines revealed apoptosis is mediated through various signaling complexes by death inducing members of tumour necrocis factor family [16]. Fas and FasL are highly expressed in hepatocytes [23]. Studies using K8 null and Wild Type mouse hepatocytes in primary culture uncovered that the K8/ K18 intermediate filament protein expressed in hepatocytes provides resistance to FAS receptor mediated apoptosis by induction of Jo2 injection which is similar to FAS ligand in K8 null mouse hepatocyte and highly sensitive wild type hepatocyte mouse models and also concluded that Fas mediated apoptosis is regulated through the interaction of K8/K18 intermediate filament proteins with the microtubule cytoskeletal protein for the trafficking of FAS receptors to the surface membrane and also trimerization of Fas receptor for Fas ligand binding [9].

3.4 Regulation of hemostasis

A thrombin–anti thrombin binding protein expressed on hepatocyte plasma membrane has protein sequence, electrophorectic mobility and insolubility in detergents which is highly homogenic with the cytokeratin 18. Culture labeling studies revealed the localization of cytokeratin 18 receptor on plasma membrane of hepatocyte. The introduction of anti-cytokeratin 18 IgG antibodies to hepatoma cell culture revealed the inhibition of radio-Iodine labelled Thrombin-anti thrombin (125I-TAT) protein complex binding and their internalization to the hepatoma cells. Low density lipoprotein receptor- related Protein is one of the surface membrane protein that interacts with the serpin enzyme complex to

eliminate thrombin-anti thrombin protein from circulation [15]. The study suggested that the initiation of Thrombin- anti thrombin binding to hepatocytes is mediated by Cytokeratin 18 acts as a cofactor which then results in the initiation of Low density lipoprotein receptor-related Protein for removal of Thrombin- antithrombin complex from circulation inorder to maintain hemostasis [30].

3.5 Regulation in protein synthesis

Cytokeratin 8 null mouse model (*ck8-/-*) studies revealed that lack of cytokeratin 8 results in absence of cytokeratin 18 which leads to improper organization of microtubules to the apical anchoring sites in the plasma membrane in hepatocytes, indicating that intermediate filaments cytokeratin 8/18 has direct influence on the the apical compartmentalization of apical membrane proteins, microtubules and γ-tubulins in polarized epithelial cell [1, 25]. *In vitro* studies using mouse model KtyII-/- revealed that protein synthesis is regulated through another pathway by keratin 8/18 filaments in simple epithelial cells. The KtyII-/- mouse that lacked the keratin 18/8 filaments in the cytoplasm showed mislocalization of GLUT 1 and GLUT 3 in the cell membrane without showing any change in the microtubule organization indicating that lack of keratins has direct influence on GLUT 1 and GLUT 3 localization which leads to phosphorylation of raptor by AMP kinase which results in the inhibition of mTOR pathway mediated protein synthesis. The KtyII-/- mouse model revealed that the keratin 8/18 expression directly regulates the apical proteins, γ-tubulins and microtubules localization and by indirect/ direct means on 14-3-3 σ proteins, AMPK, eEF 1γ which are involed in the mTOR pathway mediated protein synthesis there by regulating AMPK mediated glucose homeostasis [29, 11, 13].

4. Cytokeratin 18 and disease condition

In vitro studies using transgenic mice models that has cytokeratin 18 caspase digestion resistant double mutant K18-D238/379 in a k18 null mouse revealed that cytokeratin 18 filaments are involved in cell stability and organization, provides resistence to apoptosis that are mediated by tumour necrosis factor family [32]. The knockout mouse models show cases the role of k8 and k18 in cytoplasmic hyaline inclusion bodies, Mallory Denk Bodies formation in hepatocytes. Keratin8, 18 fragments, chaperones, phosphoepitopes, ubiquitinization products [26] are the components of Mallory Denk Bodies identified with the DDC intoxicated mice models. Mallory denk bodies serve as histological marker in liver diseases such as non-alcoholic fatty liver diseases [33]. Experiments with Kl8/K8 mutant models are more susceptible to fas mediated apoptosis when treated with toxic agents [27].

5. Role as potential biomarker

K8 is resistant to apoptosis due to conserved caspase cleavage sites, but k18 has two caspase cleavage sites VEVD238 and DALD397 which gets digested during apoptosis [17]. The fragments of apoptosis in circulation can be used as biomarker for the detection of hepatic dysfunction. Non alcoholic steatohepatitis is a chronic liver disease in humans. Liver biopsy is a gold standard test for the detection of non-alcoholic steatohepatitis. Several studies were conducted to identify a non invasive method to detect the liver injury. Hepatocyte apoptosis is the predominant feature of Non alcoholic Steatohepatitis and the degree of apoptosis depends upon the progressionof the disease. Studies were conducted to measure the levels of cytokeratin 18 fragments in plasma of patients with Non alcoholic Steatohepatitis and were compared with normal and liver biopsy studies. Results revealed that plasma levels of cytokeratin 18 fragments were significantly elevated than control group suggesting that cytokeratin 18 fragments in plasma could be used as potential biomarker in diagnosing the various states of hepatic dysfunction [7].

Mouse models with intestinal disease and hepatosplenic schistosomiasis studies showed the cytokeratin 18 levels in serum were elevated in case of hepatosplenic schistosomiasis than in intestinal disease. It showed significant changes in the levels of cytokeratin 18 in sera samples that could be correlated closely with the progression of disease [22].

Meta-Analysis of correlation of cytokeratin 18 levels in serum with hepatitis revealed that cytokeratin 18 fragments in circulation could be a detector for hepatic fibrosis and hepatoma. Studies revealed that it is a promising marker for the identification of various stages of hepatic dysfunction [31].

During apoptosis, caspases cleaves the highly conserved aspartate residues via intrinsic or extrinsic pathway. The apoptotic cleavage of cytokeratin 18 at 396 DALD site results in the neoepitope formation. The level of this neoepitope in the serum of critically ill patients was measured by using M30 antibody in circulation. The result revealed a significant elevation in the levels of M30 serum (M30>250.8 U/L) in progression with the dysfunction of liver, concluding that the elevated levels of apoptosis mediated keratin intermediate filament fragment M30 can be used as a prognostic indicator for short term mortality in critically ill patients [14].

6. As biomarker in veterinary medicine

Due to negative energy balance in dairy cows during postpartum period, lipolysis occurs to meet out the energy demand. The excess fat is re-esterified into triglycerides resulting in fatty liver disease. Lipotoxicity due to fatty liver causes oxidative stress leading to liver apoptosis. In ketotic cows the elevated levels of serum M30 concentrations were estimated to indicate the hepatic apoptosis [5].

Studies conducted in using small intestine of porcine revealed the expression of cytokeratin 18 immediately above the Payer's patches indicating that cytokeratin 18 can be used as M cell marker [8].

7. Conclusion

Cytokeratin 18 being an integral part of hepatocyte, acts as a modulator for various cellular functions of the liver and could be used as potential biomarker for various hepatic diseases.

Studies in Veterinary Medicine on the role of cytokeratin 18 as a biomarker in hepatic diseases is meager, limelight has to be shown on cytokeratin 18 in the field of veterinary medicine to improve the diagnostic approach in hepatic disorders in animals to come with fruitful outcome to safeguard the lives of animals.

8. References

1. Ameen NA, Figueroa Y, Salas PJ. Anomalous apical plasma membrane phenotype in CK8-deficient mice indicates a novel role for intermediate filaments in the polarization of simple epithelia. Journal of cell science. 2001; 114(3):563-575.
2. Caulin C, Salvesen GS, Oshima RG. Caspase Cleavage of Keratin 18 and Reorganization of Intermediate Filaments during Epithelial Cell Apoptosis. The Journal of Cell Biology. 1997; 138(6):1379-1394.

3. Chou CF, Omary MB. Mitotic arrest with anti-microtubule agents or okadaic acid is associated with increased glycoprotein terminal GlcNAc's. J Cell Sci. 1994; 107(7):1833- 43.
4. Coulombe PA, Omary MB. Hard and soft principles defining the structure, function and regulation of keratin intermediate filaments. Current Opinion in Cell Biology. 2002; 14:110-122.
5. Du X, Cheng L, Huang D, Peng Z, Zhao C, Zhang Y *et al*. Elevated Apoptosis in the liver of Dairy Cows with Ketosis. Cell Physiol Biochem. 2007; 43:568-578.
6. Feldmann G. The cytoskeleton of the hepatocyte: structure and functions. Journal of hepatology. 1989; 8(3):380-386.
7. Feldstein AE, Wieckowska A, Lopez AR, Liu NN, Zein YC, McCullough AJ. Cytokeratin 18 Fragments levels as Non invasive Biomarkers for Nonalcoholic Steatohepatitis: a Multicenter Validation Study. Hepatology. 2009; 50(4):1072-1078.
8. Gebert A, Rothkotter HJ, Pabst R. Cytokeratin 18 is an M cell marker in porcine Payer's Patches. Cell Tissue Res. 1994; 276:213-221.
9. Gilbert S, Loranger A, Daigle N, Marceau N. Simple epithelium keratins 8 and 18 provide resistance to Fas-mediated apoptosis. The protection occurs through a receptor- targeting modulation. J Cell Biol. 2001; 154(4):763-74
10. Herrmann H, Strelkov SV, Burkhard P, Aebi U. Intermediate filaments: primary determinants of cell architecture and plasticity. J Clin. Invest. 2009; 119:1772-1783.
11. Kellner JC, Coulombe PA. Keratins and protein synthesis: the plot thickens. J Cell Biol. 2009; 187(2):157-159.
12. Kim S, Coulombe PA. Intermediate filament scaffolds fulfill mechanical, organizational, and signaling functions in the cytoplasm. Genes & development. 2007; 21(13):1581-97.
13. Kim S, Kellner J, Lee CH, Coulombe PA. Interaction between the keratin cytoskeleton and eEF1Bγ affects protein synthesis in epithelial cells. Nature Structural and Molecular Biology. 2007; 14(10):982.
14. Koch A, Yagmur E, Linka J, Schumacher F, Bruensing J, Buendgens L *et al*. High Circulating Caspase-Cleaved Keratin 18 Fragments (M30) Indicate Short-Term Mortality in Critically Ill Patients. Hindawi Disease Markers. 2018; 8583121:1-8.
15. Kounnas MZ, Church FC, Argraves WS, Strickland DK. Cellular Internalization and Degradation of Antithrombin III-Thrombin, Heparin Cofactor II-Thrombin, and-Antitrypsin-Trypsin Complexes Is Mediated by the Low Density Lipoprotein Receptor- related Protein. Journal of Biological Chemistry. 1996; 271(11):6523-9.
16. Krammer PH. CD95 (APO-1/Fas)-mediated apoptosis: live and let die. In Advances in immunology. 1998; 71:163-210.
17. Ku NO, Omary MB. Effect of mutation and phosphorylation of type I keratins on their caspase-mediated degradation. Journal of Biological Chemistry. 2001; 276(29):26792-8.
18. Ku NO, Omary MB. Identification of the major physiologic phosphorylation site of human keratin 18: potential kinases and a role in filament reorganization. The Journal of Cell Biology. 1994; 127(1):161-71.
19. Kulesh DA, Oshima RG. Complete Structure of the gene for Human Keratin 18. Genomics. 1998; 4:389-347.
20. Liao J, Omary MB. 14-3-3 proteins associate with phosphorylated simple epithelial keratins during cell cycle progression and act as a solubility cofactor. The Journal of cell biology. 1996; 133(2):345-57.
21. Loranger A, Duclos S, Grenier A, Price J, Wilson-Heiner M, Baribault H *et al*. Simple epithelium keratins are required for maintenance of hepatocyte integrity. The American journal of pathology. 1997; 151(6):1673
22. Manivannan B, Rawson P, Jordan TW, Karanja DM, Mwinzi PN, Secor WE *et al*. Identification of cytokeratin 18 as a biomarker of mouse and human hepatosplenic schistosomiasis. Infection and immunity. 2011; 79(5):2051-8.
23. Nagata S. Fas ligand-induced apoptosis. Annual review of genetics. 1999; 33(1):29-55.
24. Omary MB, Ku NO, Toivola DM. Keratins: Guardians of the Liver. Hepatology. 2001; 35(2):251-257.
25. Oriolo AS, Wald FA, Canessa G, Salas PJ. GCP6 binds to intermediate filaments: A novel function of keratins in the organization of microtubules in epithelial cells. Molecular biology of the cell. 2007; 18(3):781-94.
26. Rogel MR, Jaitovich A, Ridge KM. The role of the Ubiquitin Proteosome Pathway in Keratin Intermediate Filament Protein Degradation. The American Thoracic Society. 2010; 7:71-76.
27. Strnad P, Stumptner C, Zatloukal K, Denk H. Intremediate filament cytoskeleton of the liver in health and disease. Histochem cell Biol. 2008; 129:735-749.
28. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995; 267(5203):1456-62.
29. Vijayaraj P, Kroger C, Reuter U, Windoffer R, Leube RE, Magin TM. Keratins regulate protein biosynthesis through localization of GLUT1 and-3 upstream of AMP kinase and Raptor. The Journal of cell biology. 2009; 187(2):175-84.
30. Wells MJ, Hatton MWC, Hewlett B, Podor TJ. Cytokeratin 18 is expressed on the Hepatocyte Plasma Membrane surface and interacts with thrombin-Antithrombin complexes. The journal of Biological chemistry. 1997; 272(45):28574-28581.
31. Yang ZH, Yang SX, Qin CZ, Chen YX. Clinical values of Elevated Serum Cytokeratin 18 levels in Hepatitis: A Meta-Analysis. Hepat Mon. 2015; 15(5):25328.
32. Yoon HA, Yoon SY, Hong JH, Ku NO. A Mutation in keratin 18 that causes caspase- digestion resistence protects homozygous transgenic mice from hepatic apoptosis and injury. Journal of Cell Science. 2017; 130:2541-2550.
33. Zatloukal K, French SW, Stumptner C, Strnad P, Harada M, Toivola DM *et al*. From Mallory to Mallory-Denkbodies: What, how and why?. Experimental Cell Research. 2007; 313:2033-2049.