Experimental and Molecular Pathology 104 (2018) 45–49

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Alcoholic hepatitis versus non-alcoholic steatohepatitis: Levels of expression of some proteins involved in tumorigenesis

Luan Nguyen[a,*], Maryam Masouminia[a], Alejandro Mendoza[a], Sara Samadzadeh[a], Brittany Tillman[a], Timothy Morgan[b], Barbara French[a], Samuel French[a]

[a] Harbor-UCLA Medical Center, Torrance, CA, United States
[b] VA Medical Center, Long Beach, CA, United States

ARTICLE INFO

Keywords:
Tumorigenesis
Alcoholic hepatitis
Pre-cancer gene expression
Hepatocellular carcinoma (HCC)

ABSTRACT

Non-alcoholic steatohepatitis (NASH) is commonly associated with obesity, type 2 diabetes, and/or hypertriglyceridemia, while alcoholic steatohepatitis (ASH) is associated with alcohol abuse. Both NASH and ASH patients can develop cirrhosis and hepatocellular carcinoma (HCC) if left untreated. However, the rate of tumorigenesis in NASH and ASH appears to be different. Individuals with NASH progress to HCC at a rate of 0.5% annually (Lindenmeyer and McCullough, 2018), when individuals with ASH progress to HCC at a rate of 3–10% annually (Schwartz and Reinus, 2012). Thus, the objective of our study is to determine if there are differences in NASH versus ASH in the levels of different proteins expressed involved in cancer development. The method used was measuring the proteins expressed in liver biopsied sections from NASH and ASH patients using immunohistochemical staining with fluorescent antibodies and then quantitating the fluorescence intensity morphometrically. The 20 proteins tested are parts of the Ingenuity Canonical Pathway of Molecular Mechanisms of Cancer and include: RAP2B, NAIP, FYN, PAK6, SUV39H1, GNAI1, BAX, E2F3, CKDN2B, BAK1, BCL2, DIABLO, RASGRF2, GNA15, PIK3CB, BRCA1, MAP2K1, BIRC3, CDK2, and ATM. In ASH, the proteins that showed upregulated levels of expression were SUV39H1, E2F3, BCL2, BAK1, BIRC3, and GNAI1. In NASH, the proteins that showed upregulated levels of expression were BAK1 and GNAI1 and the protein that showed downregulated level of expression was BCL2. Additionally, levels of expression for SUV39H1, E2F3, BCL2, BAK1, BIRC3, and GNAI1 were significant upregulated in ASH compared to NASH. These results showed significant differences in ASH compared to normal liver, and significant differences in ASH compared to NASH. Thus, we conclude that there are more proteins involved in tumorigenesis in ASH compared to NASH and in ASH compared to normal liver, which is consistent with the known tumor development rate in ASH and NASH.

1. Introduction

Molecular Mechanisms of Cancer Pathway is one of the Ingenuity Canonical Pathways developed by Qiagen Corporation (Table 1) (Liu et al., 2015a, 2015b). These pathway categories are adopted by science research communities world-wide in the quest to uncover cancer pathogenesis. In this particular pathway, the 20 proteins involved are RAP2B, NAIP, FYN, PAK6, SUV39H1, GNAI1, BAX, E2F3, CDKN2B, BAK1, BCL2, DIABLO, RASGRF2, GNA15, PIK3CB, BRCA1, MAP2K1, BIRC3, CDK2, and ATM. In our study, six of these proteins showed significant differences in protein levels' expression between ASH and NASH and control liver. These proteins are discussed below.

SUV39H1 is a histone methyltransferase involved in regulating transcription and promoting cell growth. SUV39H1 overexpression plays important roles in HCC development and progression (Chiba et al., 2015). SUV39H1 and ESET function to methylate Histone H3 to allow it to progress with transcription through formation of histone H3 lysine 9 trimethylation (H3K9me3). Interestingly, only SUV39H1 knockdown, but not ESET knockdown, reduces H3K9me3 levels and impairs HCC cell growth and sphere formation. Thus, SUV39H1 could be a potential pharmacological inhibition target in preventing hepatocellular carcinoma development.

E2F3 is a member of the E2F family of transcription factors and plays a crucial role in the control of cell cycle. E2F3's two cousins E2F1 and E2F2 are negatively regulated by pRb, a well-known tumor suppressor. Myc, a popular onco-protein, activates E2F3. E2F3's copy number gains are frequently observed in HCC and almost all HCC samples have increased expression of E2F3 (Kent et al., 2017).

BCL2 inhibits apoptosis along the intrinsic mitochondrial apoptosis pathway. BCL2 is part of a large family of proteins involved both in

* Corresponding author.
E-mail address: lnguyen23289@yahoo.com (L. Nguyen).

https://doi.org/10.1016/j.yexmp.2017.12.007
Received 22 December 2017; Accepted 31 December 2017
Available online 04 January 2018
0014-4800/

Table 1
Molecular mechanisms of cancer pathway (bolded).

Canonical pathways	
Ingenuity canonical pathways	Molecules
BRCA1-mediated tumor suppression	CDNK1A,FANCG,BRCA2,SLC19A1,BRCC3,RFC5,E2F3,BRCA1,FANCA,ATM
Cell Cycle: G1/S checkpoint regulation	NRG1,HDAC11,SUV39H1,E2F3,CDKN1A,CDKN2B,CDK2,ATM
p70S6K signaling	PLCD3,CD19,IL2RG,SYK,GNAI1,PIK3CB,SFN,PLCL1,MAP2K1,ATM
Tec kinase signaling	TEC,FYN,STAT5A,GNG11,PAK6,GNA15,GNG2,GNAI1,STAT2,PIK3CB,FRK,ATM
DNA double-strand break repair by homologous recombination	BRCA2,BRCA1,ATM
Apoptosis signaling	DIABLO,NAIP,BAX,TNFRSF1B,BIRC3,MAP2K1,BAK1,BCL2
Fatty acid α-oxidation	BCO2,PTGS2,ALDH3A1
CCR3 signaling in eosinophils	GNG11,PAK6,GNG2,PLA2G5,GNAI1,PIK3CB,LIMK2,MAP2K1,ATM
Prostate cancer signaling	SUV39H1,PIK3CB,CREB5,MAP2K1,CDK2,ATM,BCL2
p53 signaling	SIRT1,PIK3CB,BAX,SFN,BRCA1,CDK2,ATM,BCL2
GADD45 signaling	BRCA1,CDK2,ATM
IL-9 signaling	STAT5A,IL2RG,PIK3CB,ATM
IL-8 signaling	NOX4,GNG11,FLT4,GNG2,GNAI1,PIK3CB,LIMK2,BAX,PTGS2,MAP2K1,ATM,BCL2
UVA-Induced MAPK signaling	PLCD3,PARP16,PIK3CB,RPS6KA5,PLCL1,SMPD3,ATM
Interferon signaling	STAT2,BAX,BAK1,BCL2
Role of tissue factor in cancer	FYN,STAT5A,GNA15,PIK3CB,RPS6KA5,LIMK2,FRK,ATM
Molecular mechanisms of cancer	**RAP2B,NAIP,FYN,PAK6,SUV39H1,GNAI1,BAX,E2F3,CDKN2B,BAK1,BCL2,DIABLO,RASGRF2,GNA15,PIK3CB,BRCA1,MAP2K1,BIRC3,CDK2,ATM**
Natural killer cell signaling	FYN,PAK6,SYK,PIK3CB,HCST,MAP2K1,INPP5D,ATM
G-protein coupled receptor signaling	FYN,PTGIR,PDE3A,GNAI1,PDE1A,CREB5,HRH1,GNA15,ADRA2A,PDE4D,DUSP4,PIK3CB,MAP2K1,ATM,HTR2A

Bold defines the particular pathway of interest in this research.

preventing apoptosis and promoting it. BCL2 protects mitochondrial cell membranes in the events of cytotoxic injuries. Bcl-2 may play a role in hepatocarcinogenesis as an inhibitor of apoptosis of tumor cells (El-Emshaty et al., 2014).

BAK1 is a member of the BCL2 protein family that functions as a pro-apoptotic regulator. BAK and BAX form homo-oligomers within the mitochondrial membrane, resulting in the release of cytochrome c, which activates Apaf1 and results in caspase 9 activation. BAK1 works with other pro-apoptotic molecules in the mitochondria, including DIABLO (Cory and Adams, 2002). BAK1's upregulation induces apoptosis of HCC cells. (A et al., 2011).

BIRC3 is a member of the inhibitor of apoptosis (IAP) family of proteins. BIRC3 inhibits apoptosis by binding to tumor necrosis factor receptor-associated factors TRAF1 and TRAF2. BIRC3's DNA amplification has been observed in mouse HCC (Zender et al., 2006).

GNAI1 functions as a transducer downstream of G protein-coupled receptors in numerous signaling cascades. GNAI1 can regulate cell proliferation and differentiation, assist platelet aggregation, and act as receptors in multiple cancers. GNAI1 is significantly down-regulated in HCC compared with normal liver (Yao et al., 2012). GNAI1 is hypothesized to function as an inhibitor of HCC migration and invasion.

The other 14 proteins' functions and significance are summarized in Table 2.

2. Methods

Formalin-fixed paraffin-embedded biopsies of 8 to 12 alcoholic hepatitis livers, 1 to 5 NASH livers, and 3 normal livers were obtained from Harbor-UCLA Medical Center and from the Long Beach Veterans Affairs' clinical trial in treatment of alcoholic hepatitis. The study was carried out according to the principals of the Declaration of Helsinki and was designated as exempt by our institutional ethics review board. The data was analyzed anonymously. The slides were double stained for ubiquitin plus one of the twenty proteins tested using a fluorescent labeled antibody. Texas Red (Millipore, Temecula, CA) was used to detect ubiquitin. Either donkey-anti mouse or anti rabbit Alex Fluor (Jackson Labs, West Grove, PA) were used as the second antibody to detect the protein. The staining of all the samples was done at the same time to provide accurate comparison between groups.

We measured the intensity of the fluorescent staining in three different areas on each slide with 40 × magnifications and 800 ms standard exposure time by using a Nikon 400 fluorescent microscope. The Nikon morphometric system was used to quantitate the florescent intensity (See Fig. 1). The mean, standard error, and statistical differences of data achieved from the Nokia were analyzed by Graph pad statistical software. Control versus ASH, control versus NASH, and ASH versus NASH were compared by unpaired *t*-test. Only $p < 0.05$ was considered statistically significant.