European Journal of Cancer 83 (2017) 56–70

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

New liver cancer biomarkers: PI3K/AKT/mTOR pathway members and eukaryotic translation initiation factors

Nicole Golob-Schwarzl [a,b], Stefanie Krassnig [a], Anna M. Toeglhofer [a], Young Nyun Park [c], Margit Gogg-Kamerer [a], Klemens Vierlinger [d], Fabian Schröder [d], Hyungjn Rhee [e], Rudolf Schicho [f], Peter Fickert [g], Johannes Haybaeck [a,b,h,*]

[a] *Department of Pathology, Medical University of Graz, Austria*
[b] *Center for Biomarker Research in Medicine, Graz, Austria*
[c] *Department of Pathology, Yonsei University, College of Medicine, Seoul, South Korea*
[d] *AIT Austrian Institute of Technology GmbH, Vienna, Austria*
[e] *Department of Pathology, Yonsei University, University College of Medicine, Seoul, South Korea*
[f] *Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria*
[g] *Division of Gastroenterology and Hepatology, Medical University of Graz, Austria*
[h] *Department of Pathology, Otto-von-Guericke-University Magdeburg, Germany*

Received 16 May 2017; accepted 1 June 2017
Available online 14 July 2017

KEYWORDS
Non-virus-related hepatocellular carcinoma;
Virus-related hepatocellular carcinoma;
Chronic hepatitis B;
Chronic hepatitis C;
Translation initiation

Abstract Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide. The initiation of protein translation is an important rate-limiting step in eukaryotes and is crucial in many viral infections. Eukaryotic translation initiation factors (eIFs) are involved in the initiation step of protein translation and are linked to the phosphatidylinositol-3-kinases PI3K/AKT/mTOR pathway. Therefore we aimed to investigate a potential role of eIFs in HCC. We herein report on the immunohistochemical expression of the various eIF subunits in 235 cases of virus-related human HCC. Additionally, we used immunoblot analysis to investigate the expression of virus-related HCC and non-virus-related HCC in comparison to controls. Mammalian target of rapamycin (or mechanistic target of rapamycin as it is known now (mTOR) and activated mTOR were significantly increased in chronic hepatitis C (HCV)-associated HCC, in HCC without a viral background, in alcoholic liver disease and Wilson disease. pPTEN, phosphatase and tensin homologue (PTEN) and pAKT showed a significant increase in HBV- and HCV-associated HCC, chronic

* *Corresponding author*: Department of Pathology, Otto-von-Guericke-University Magdeburg, Leipziger Straße 44, D-39120 Magdeburg, Germany. Fax: +49 391 67 15818.
E-mail address: johannes.haybaeck@med.ovgu.de (J. Haybaeck).

http://dx.doi.org/10.1016/j.ejca.2017.06.003

hepatitis B, HCC without a viral background, alcoholic steatohepatitis (ASH) and Wilson disease. Phosphorylated (p)-eIF2α, eIF2α, eiF3B, eIF3D, eIF3J, p-eIF4B, eIF4G and eIF6 were upregulated in HCV-associated HCC. eIF2α, p-eIF4B, eIF5 and various eIF3 subunits were significantly increased in chronic hepatitis B (HBV)-associated HCC. HCC without viral background displayed a significant increase for the eIF subunits p-2α, 3C, 3I, 4E and 4G. We noticed engraved differences in the expression pattern between chronic hepatitis B and C, HBV- and HCV-associated HCC and non-virus-related HCC.

1. Introduction

Biomarkers are indispensable for prognostic reasons and future tailored therapy.

Translation initiation is an important rate-limiting step in eukaryotic protein translation, and it is a crucial target in many viral infections. The upregulation of translation initiation in cancer plays a major role in tumorigenesis [1–16]. The initiation step involves a group of different ribosomal subunits, initiation factors and mRNA to compose an 80S ribosomal complex [1–3,16–18].

Eukaryotic translation initiation factors (eIFs) are involved in the initiation step of protein translation [3]. Each eIF fulfills a unique role in the initiation step by interacting with ribosomal subunits and mRNAs to compose an elongation-appropriate complex [18]. eIF4A, eIF4B, eIf4G, and eIF4E activating mRNA during eIF1, eIF1A, eIF2, eIF3, and eIF5 interact with 40S and 60S ribosomal subunits. Activated mRNA binds to the 40S and 60S to form the 80S ribosomal complex, which initiates translation [18,19].

The phosphatidylinositol-3-kinase PI3K/AKT/mTOR pathway is involved in the control of cell growth and proliferation and has demonstrated constitutive activation in different cancer types [3,20,21]. Deregulation in protein synthesis is a result of accumulated mutations in a cell. For instance, deregulation can be caused by aberrant expression of eIFs combined with aberrant activation of the mTOR signalling pathway [16,22,23].

Previous studies have demonstrated overexpression of eIF5A2 in various human cancers, including HCC. eIF5A2 has shown to play a major role in malignant transformation, tumour cell proliferation and development of metastasis [24–27]. Other eIFs, such as eIF4E, are strongly expressed in head and neck squamous cell carcinoma, non-small cell lung cancer, breast cancer and HCC. It can bind to the cap structure located at the 5' end of mRNA and is fundamental for mRNA translation initiation. It is a key factor in the initial stage of protein synthesis [28–30]. However, viruses are known to access and adapt the host translation machinery by a variety of mechanisms [31]. These interactions result in an upregulated viral protein synthesis and could repress host translation [32–35].

In this study, we aimed to investigate the involvement of PI3K/AKT/mTOR pathway members and various eIFs in non-virus-related HCC, chronic hepatitis B (HBV)- and chronic hepatitis C (HCV)-associated HCC, chronic hepatitis B (HBV) and C (HCV), alcoholic liver and Wilson disease. For that aim we used protein analyses, immunohistochemistry and immunoblotting to characterize the expression pattern of the PI3K/AKT/mTOR pathway members and various eIF subunits.

2. Materials and methods

2.1. Patients

This retrospective study included 235 HCC patients operated at the Department of Surgery, Yonsei University College of Medicine, Seoul, Korea.

Clinical data were available from all patients. All tumour tissues and non-neoplastic liver tissues (NNLTs) were fixed in formalin and embedded in paraffin according to routine methods. The study was reviewed and approved by the institutional ethics committee of the Medical University of Graz in agreement to Austrian and European laws (20-119 ex 08/09). Histological tumour type and grade were evaluated according to the World Health Organization (WHO) cancer classification and tumour stage according to the Union for International Cancer Control/ l'Union Internationale Contre le Cancer (UICC) TNM classification. The cohort included 196 (83.4%) male and 39 (16.6%) female patients. Age ranged between 30 and 81 years, with a median of 56 years. Disease aetiology was as listed in Table S1: chronic hepatitis B in 194 patients (82.5%), chronic hepatitis C in 12 patients (5.1%) and alcoholic liver injury in 11 patients (4.7%). Seventy-eight patients did not show cirrhosis, in comparison to 116 (60%) patients who did (Table S1). Fibrosis grade, Child-Pugh score and class were also analysed. Child-Pugh score/class is an assessment tool for prognosis and liver function of cirrhotic patients. In our patient cohort, most patients revealed Child-Pugh class A with a score of 5, indicating favourable liver functional status. Ten patients revealed no fibrosis, eight of the cohort 1. A fibrosis grade of 2 was observed in 34 patients, whereas