GPX4 and *GPX7* over-expression in human hepatocellular carcinoma tissues

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and is still one of the most fatal cancers. Hence, it needs to identify always new putative markers to improve its diagnosis and prognosis. The selenium is an essential trace mineral implicated as a key factor in the early stage of cancer and exerts its biological function through the selenoproteins. In the last years our group has been studying the involvement of some selenoproteins in HCC. However, no many data are reported in literature about the correlation between HCC and the glutathione peroxidases (GPXs), both selenium and non selenium-containing GPXs.

In this paper we have evaluated the *GPX4* and *GPX7* expression in some paraffin-embedded tissues from liver biopsy of patients with hepatitis C virus (HCV)-related cirrhosis and HCC by immunohistochemistry and RT-qPCR analysis. Our results evidenced that i) *GPX4* and *GPX7* had a statistically significant over-expression in HCC tissues compared to cirrhotic counterparts used as non tumor tissues, and ii) their expression was higher in grade III HCC tissues with respect to grade I-II samples. Therefore, we propose to use *GPX4* and *GPX7* as possible markers for improving HCC diagnosis/prognosis.

Introduction

Liver cancer is the second-leading cause of cancer mortality worldwide, accounting for approximately 600,000 cancer-related deaths annually.[1] Hepatocellular carcinoma (HCC), the most common type of liver cancer, generally develops from chronic liver injury,[2] and its risk factors are multiple, such as hepatitis B (HBV) or C virus (HCV) infection, alcohol-induced liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), primary biliary cirrhosis, exposure to environmental carcinogens (particularly aflatoxin), or even type 2 diabetes and obesity.[3] Despite recent advances in diagnosis and management, the median survival of HCC patients is less than 8 months, and the disease is still one of the most fatal cancers.[4] Surgical resection, liver transplantation, and local ablation remain the only HCC curative modalities,[5,6] and recurrence occurs in up to 70% of patients within 5 years after resection.[7,8] Unfortunately, the molecular signaling mechanisms, which specifically lead to HCC, are shielded and perturbed by molecular signaling sustained by viral infection as well as other diseases such as, for instance, diabetes. Therefore, it is necessary to identify always new putative markers to improve the HCC prognosis. Recently some Authors evaluated the expression also of other two proteins, thymosin beta 4 (Tβ4) and thymosin beta 10 (Tβ10) in HCC tissues. This study showed the expression of both beta-thymosins in HCC with marked differences in their degree of expression and frequency of immunoreactivity. The higher incidence of Tβ10 expression and its higher reactivity in tumor cells involved in stromal invasion indicated a possible major role for Tβ10 in HCC progression.[9]

Some studies evidenced the role of selenium (Se) to assist cells in resisting to oxidative damage that is a major cause of cellular damage also because it was found implicated as a key factor in the early stage of cancer.[10] *In vivo*, Se is primarily present as selenoproteins to maintain the balance of the cellular redox state, and in humans there are 25 selenoproteins.[11] In the last years our group has been focusing on some selenoproteins and their involvement in HCC, also evaluating by immunohistochemistry (IHC) the expression of selenium binding protein-1 (SELENBP1), which is, *in vivo,* able to incorporate exogenously administered radioactive (75Se)-sodium selenite in the liver, as well as that of Selenoprotein M (SELM) in tissue samples of HCC patients.[12-14] These studies provided evidence that SELENBP-1, as well as selenium, is down-regulated in the liver tissue of HCC patients and that its gradual loss is associated with an increased malignant grade.[12,13] Moreover, we showed for the first time an increase of SELM expression in HCC liver tissues, and its correlation with their increased malignancy grade.[14] Also, we have recently carried out the analysis of the global expression of the seleno-transcriptome protein family in two human hepatocellular carcinoma cell lines (HepG2 and Huh7) compared to the normal human hepatocytes by means of the RT-qPCR analysis.[15,16] These studies have shown a signature of selenoprotein mRNAs specific for human hepatoma cells showing the genes that change their expression as a consequence of the liver cancer in the absence of any genetic mutations or viral infection, and, in particular, that in HepG2 and Huh7 cells there were three down-regulated and ten over-regulated genes, among which SELM, and two glutathione peroxidases such as *GPX4* and *GPX7*.[15,16]

In general, the GPXs belong to a family of phylogenetically related enzymes, and *GPX4* and *GPX7* are strictly correlated because the Cys-containing *GPX7* is evolved from a *GPX4*-like ancestor.[17] Actually, these two proteins present a percentage of sequence identity of about 50% and the same fold topology of an alpha-beta 3-layer sandwich type.[18] *GPX4* has some role in the regulation of apoptosis, whereas *GPX7* is reported to be involved in the protein folding.[17] Also, *GPX4* was found over-regulated at the protein level in human colon carcinoma tissue and the impaired expression of its gene in peripheral blood mononuclear cells was proposed as a biomarker of increased breast cancer risk.[19,20] Further, *GPX4* has been significantly associated with breast cancer survival among the patients with the highest Native American (NA) ancestry whereas its variants resulted to be correlated with the risk of lethal prostate cancer, and able to modify the relation between γ-tocopherol and prostate cancer survival.[21,22]

Recently, *GPX4* has been found to play an essential role in the hepatitis C virus (HCV)

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Key words: Hepatocellular carcinoma; *GPX4*; *GPX7*; immunohistochemistry; RT-qPCR.

Acknowledgments: the authors thank Marilina Russo for assistance in figure preparation.

Received for publication: 5 June 2015.
Accepted for publication: 7 October 2015.

European Journal of Histochemistry 2015; 59:2540
doi:10.4081/ejh.2015.2540