Tissues, Pathology, and Diagnostic Microscopy

LS.2.P047
Analysis of epidermal growth factor receptor (EGFR) expression in hepatocellular carcinoma; a comprasion of fluorescence in situ hybridization (FISH) and immunohistochemisrty (IHC)

S. Uslu[1], H. Kirimlioglu[2], U. Ince[2]

[1]Acibadem University, Vocational school of health services pathology laboratory technician program, Istanbul, Turkey
[2]Acıbadem university, pathology, ıstanbul, Turkey

musiuslu@gmail.com
Keywords: Hepatocellular carcinoma, epidermal growth factor receptor, fluorescence in situ hybridization, immunohistochemistry

Hepatocellular carcinoma (HCC) is a poor prognostical tumor. Though it is a leading cause of death, the conventional systemic therapy strategies are not effective. In recent studies the expression of EGFR in the majority of HCCs makes it a promising target of anti-EGFR therapies. In our study, we aimed to demonstrate and compare the EGFR gene aberration by fluorescence in situ hybridization (FISH) and immunohistochemical (IHC) method, to determine if it can be used practically. Several studies have demonstrated positive immunoexpression of cytokeratin (CK) 19 in HCC, and CK19-positive HCC has a high metastatic potential, which is also associated with a poor prognosis. Recent studies indicate that, the activation of the EGF–EGFR signaling pathway is associated with the development of CK19-positive HCC, and the EGF-induced increase in growth abilities of HCC, may account for the poor prognosis of the patients (1). We also aimed to demonstrate the relation between established prognostic features, like tumor differentiation, vascular invasion with the CK-19 immuohistochemical positivity. Twenty-six patients with hepatocellular carcinoma (HCC) without having any metastasis and recurrences clinically at the time of tumor resection were recruited in our study. The age, gender, chronic liver disease and the size of the tumor were extracted from clinical and pathologic in records. Five-micrometer thick sections were cut from paraffin blocks and were stained with hematoxyline and eosin (H&E) The pattern of the tumor, presence of clear cell change, steatosis, giant cell formation, tumor necrosis and tumor grade were noted. Immunohistochemistry for EGFR and CK19 was performed on three–micrometer thick serial sections by streptoavidin-biotin method utulizing Ventana Benchmark Ultra automated stainer (Arizona, USA).To the five-micrometer thick sections were used for FISH analyses by The SPEC EGFR/CEN 7 dual color probe and the SPEC EGFR/CEN 7 dual color probe kit (ZytoLight, ZytoVision GmbH, Germany). After the FISH procedure, the slides were examined on an Olympus Bx50 microscope and photographed with an Olympus Dp20 camera. One hundred non-overlapping nuclei were counted by a histologist in a double-blind manner. No amplification: 1-5 copies of the gene present per nucleus in >50% cancer cells, low amplification: 6-10 copies of the gene, or a small gene cluster, present per nucleus >50% cancer cells, amplification >10 copies, or large clusters, of the gene present per nucleus in >50% cancer cells (2,3).

In our study although there was correlation with vascular invasion ($p<0.01$) we could not find any association between gene expression of EGFR and IHC expression of CK19 positivity, however tumor size, chronic hepatitis etiology, tumor pattern presence of clear cell change, steatosis, giant cell formation, tumor necrosis and tumor grade ($p>0.05$). We have shown that the IHC expression of EGFR in HCC is not related to EGFR gene copy number. Contrary to other studies, we could not identify EGFR gene amplification in our cases by FISH. IHC expression of EGFR gene, made up 42% of our HCC cases, which coincides with the results of the study of Buckley et al. and our study, the IHC expression of EGFR supported that, the EGFR is certainly associated with the development of HCC (4). In our study none of our cases had either metastasis or recurrence which was found to be different from the previous studies (4). In contrary to the previous studies, we could neither find any statistically significant amplification by FISH method, nor any strong EGFR expression by IHC analysis, which may be attributable to the early stage of the HCC cases. However, the nuances, compared with the other studies suggested that, the EGFR may also pose a role of the HCC progression. As there is considerable disparity in the literature about the relationship of EGFR expression and prognostic features in HCC further studies of larger series are needed.

1. Yoneda N, Sato Y, Kitao A, Ikeda H, Sawada-Kitamura S, Miyakoshi M, Harada K, Sasaki M, Matsui O, Nakanuma Y. Epidermal growth factor induces cytokeratin 19 expression accompanied by increased growth abilities in human hepatocellular carcinoma. Laboratory Investigation 2011; 91, 262–272.
2. Bernardes VF, Gleber-Netto FO, Sousa SF, Rocha RM, Aguiar MCF. EGFR status in oral squamous cell carcinoma: comparing immunohistochemistry, FISH and CISH detection in a case series study. BMJ open accessible medical research 2013;3:e002077. Doi:10.1136/bmjopen-2012-002077.
3. Shia J, Klimstra DS, Li AR, Qin J, Saltz L, Teruya-Feldstein J, Akram M, Chung KY, Yao D, Paty PB, Gerald W, Chen B. Epidermal growth factor receptor expression and gene amplification in colorectal carcinoma: an immunohistochemical and chromogenic in situ hybridization study. Modern Pathology 2005:18;1350-1356.
4. Anne F. Buckley, MD PhD,1 Lawrence J. Burgart, MD,2 Vaibhav Sahai,3 and Sanjay Kakar, MD3. Epidermal Growth Factor Receptor Expression and Gene Copy Number in Conventional Hepatocellular Carcinoma. Am J Clin Pathol 2008;129:245-251.

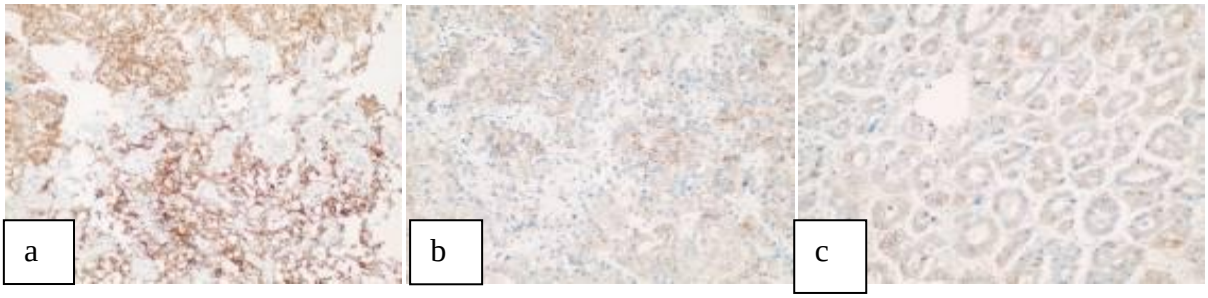

Figure 1. Immunohistochemical analysis for epidermal growth factor receptor showing strong membrane expression (a), moderate membrane expression (b), mild expression (c) in hepatocellular carcinoma (x40).

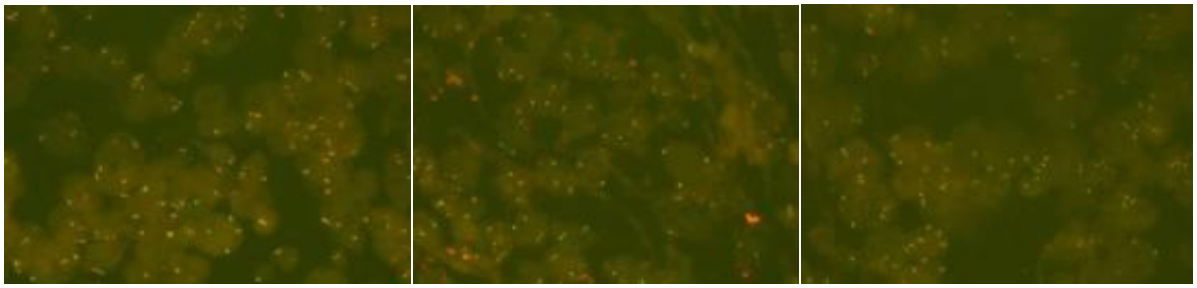

Figure 2. Fluorescence in situ hybridization using probes directed against the epidermal growth factor receptor (EGFR) gene (green) and centromere of chromosome 7 (red) (x1000).