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SHORT COMMUNICATION

ERCC1 is not expressed in hepatocellular cancer: A Turkish Oncology Group, Gastrointestinal Oncology Subgroup study

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Summary

Purpose: *Hepatocellular cancer (HCC) is a common malignancy with a high mortality rate. Existence of excisional repair cross complementation1 (ERCC1) is implicated in resistance to cisplatin treatment. Expression of ERCC1 in HCC is not known. In this study we aimed to find out whether a subset of HCC patients can be identified to benefit from cisplatin.*

Methods: *Sixty-one patients with HCC who had enough tissue to do immunohistochemistry were identified in 3 institutions. Immunohistochemical staining was performed manually using the standard streptavidin-biotin-peroxidase method. Monoclonal anti-ERCC 1 (D-10) antibody from Santa Cruz Biotechnology (Santa Cruz, CA) was used.*

Results: *Only one out of 61 patients (1.6%) had ERCC1 expression.*

Conclusion: *Although around 10% of HCC patients respond to cisplatin, this is unlikely to be due to ERCC1 negativity. Pathways other than ERCC1 should be searched to find ways to help these patients' treatment strategies.*

Key words: ERCC1, hepatocellular cancer, immunohistochemistry

Introduction

HCC is the 6th most common cancer worldwide [1]. Southern Europe where Turkey geographically belongs has the highest incidence of HCC comparing to other parts of Europe [2]. Although male predominance and the incidence rates are consistent with the world literature, HCC is not one of the top 10 cancers seen in Turkey [3]. The 3-year actuarial survival in advanced cases is at a dismal 8% and this requires novel approaches to treatment of this disease [4]. The multifactorial pathogenesis of this cancer makes it more amenable to interventions at several levels. Many combinations of cytotoxic drugs and targeted therapies have been studied and additional studies are ongoing as well but so far no significant gain has been reported beyond sorafenib.

ERCC1 is a DNA repair enzyme and noted to be negative prognostic indicator in cisplatin-treated lung cancer patients in the first part of this decade [5]. A subset of the IALT study showed that most adjuvant cisplatin-related benefit was restricted to ERCC1-negative patients. These findings were confirmed in the recent 8-year follow up [6]. Similar findings are also reported for esophageal and gastric cancers as well but these results are less conclusive because of size and power [7].

The ERCC1 expression in HCC is not known. Approximately 10% of patients with HCC respond to cisplatin. This subset of patients may be an ERCC1-negative subset of HCC. If this could be proven with HCC then a "tailored" treatment approach for this subset of patients could be possible.

Methods

Rarely HCC patients present in resectable stage. In advanced cases the diagnosis of HCC is made on clini-

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cal grounds and histology is confirmed with aspiration biopsy only. Therefore it may be difficult to find HCC tumor blocks with enough tissue for immunohistochemistry. Three institutions (two from Istanbul and one from Kayseri) gathered 61 HCC cases together. Since we did not know the outcome of the cases, no attempt was made at this stage to find out the patient characteristics. But we expected these patients to have 2 to 1 male predominance and median age of early 60s. All patients whose tumor block was available were included to the study.

Immunohistochemistry

The paraffin-embedded samples were fixed in 10% neutral-buffered formalin. Immunohistochemical staining was performed manually using the standard streptavidin-biotin-peroxidase method. Serial sections of 3-µm thickness were cut from the paraffin blocks and placed on Polysine™ (Menzel) slides. The sections were dried at 60°C in an oven for 1 h, deparaffinized in xylene and then dipped in ethyl alcohol. Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide/methanol. To unmask the antigens, the slides were then microwave-treated in 10 mM citrate buffer (pH 6.0). Tissue sections were rinsed with PBS and nonspecific protein binding was blocked using goat serum. Monoclonal anti-ERCC 1 (D-10) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a dilution of 1:50. Sections were incubated with anti-ERCC 1 at room temperature for 1 h followed by biotinylated goat anti-mouse IgG (Santa Cruz, CA) and peroxidase-conjugated streptavidin (Santa Cruz, CA). Staining was visualized with 3.3'-diaminobenzidine (Santa Cruz, CA). The slides were counterstained with Mayer's hematoxylin. The positive control for ERCC 1 was a breast carcinoma.

Results

Only the nuclear staining was considered as true positive and only one out of 61 (1.63%) cases expressed ERCC1. Because several staining attempts failed and the pathologists were not familiar with this particular stain we contacted the manufacturer about the stages of the staining process to make sure no technical problem is causing a false negative stain. The control breast carcinoma staining was carried out all along without a problem.

Discussion

In this study, we assessed ERCC1 expression in HCC. Cisplatin response to HCC treatment is dismal and ranges between 10-17% [8].

We hypothesized that the low response to cisplatin in HCC patients could be attributed to high ERCC1 levels in HCC cells, but instead, ERCC1 expression rate in our study group was found extremely low (1.6%). To our knowledge this is the largest study in the literature looking for ERCC1 expression in HCC.

Our hypothesis was that if the ERCC1-negative subset was only 10-15% in HCC patients then we would raise the question that maybe the cisplatin-sensitive HCC patients are the ones with ERCC negativity as is the case with non small cell lung cancer (NSCLC) patients. But 98% negativity rate makes this hypothesis invalid. Obviously there are many pathways involved in cisplatin resistance, such as multidrug resistance-associated proteins [9]. Search of these pathways' involvement may pave the way for "tailored" treatment options that may be combined with sorafenib.

One plausible explanation for the low expression of ERCC1 could be that loss of ERCC1 expression in hepatocytes may be one of the leading factors for genetic instability, and thus of tumorigenesis [10]. Impaired nucleotide excision repair pathway, in which ERCC1 plays a major role, leads to reduced DNA repair capability and increases DNA adduct levels. In fact, reduced ERCC1 expression compared to cancer-free controls has been demonstrated in other types of cancer [11].

A limited number of studies in the literature have assessed ERCC1 in HCC by DNA or RNA analysis. Protein expression assessed directly by immunohistochemistry rather than mRNA analysis may be more appropriate. Polymorphisms in codons encoding ERCC1 might influence its expression, although this change may remain undetected in the level of mRNA synthesis [12].

There are some weak points of this study. It would have been better if mRNA analysis was performed, which could have provided an explanation for the low ERCC expression in HCC. Furthermore, if mRNA analysis was performed ERCC1 polymorphisms could have been identified and further explanation for cisplatin resistance in HCC could have been provided. Other limitations are the subjectivity of immunohistochemical staining, its semiquantitative nature, the effects of tissue aging, and inter-observer variation. Despite the mentioned weak points, our study is the largest that investigated the expression rate of ERCC1 in HCC in the literature.

Conclusion

ERCC1 expression in HCC is very low. Low response rate to cisplatin in HCC patients could not be

explained with the lack of ERCC1 expression alone. Since many pathways are involved in the pathogenesis of this disease other pathways should be explored. Low ERCC1 expression may be a leading causative factor in tumorigenesis of HCC.

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