Aberrant expression of cancer stem cell markers (CD44, CD90, and CD133) contributes to disease progression and reduced survival in hepatoblastoma patients: 4-year survival data

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Hepatoblastoma (HB) is an embryonal tumor of the liver in children. Prognosis and response to treatment in HB are highly variable. Cancer stem cells (CSCs) constitute a population of cells, which contribute to the development and progression of many tumors. However, their role in HB is not well defined yet. We assessed the prognostic and predictive values of some CSC markers in HB patients. Protein and messenger RNA expressions of the CSC markers *CD133*, *CD90*, and *CD44* were assessed in 43 HB patients and 20 normal hepatic tissues using immunohistochemistry and quantitative real-time polymerase chain reaction. The expression levels of these markers were correlated to standard prognostic factors, patients' response to treatment, overall survival (OS), and disease-free survival (DFS). *CD44*, *CD90*, and *CD133* proteins were detected in 48.8%, 32.6%, and 48.8% compared with 46.5%, 41.7%, and 58.1% RNA, respectively (concordance, 77.8%–96%). None of the normal tissue samples was positive for any of the markers. Significant correlations were reported between α-fetoprotein and both *CD44* and *CD133* ($P = 0.02$) as well as between tumor types *CD90* and *CD133* ($P = 0.009$). Reduced OS correlated with *CD44*, *CD90*, and *CD133* expressions ($P < 0.001$), advanced stage ($P < 0.001$), response to treatment ($P < 0.001$), and total excision of the tumor. Reduced DFS correlated with *CD44* and *CD133* expressions ($P < 0.001$) only. In conclusion, *CD133*, *CD44*, and *CD90* could be used as prognostic and predictive markers in HB. High expression of these markers is significantly associated with poor response to treatment and reduced survival. Moreover, complete surgical resection and systemic chemotherapy are essential to achieve good response and prolonged survival, especially in early stage patients. (Translational Research 2015;165:396–406)

Abbreviations: ABC = ATP-binding cassette; AFP = Alpha fetoprotein; COG = Clinical Oncology Group; CR = Complete response; CSCs = Cancer stem cells; CT = Computerized tomography; DFS = Disease free survival; DP = Disease progression; GPI = Glysyl-phosphatidyl-inositol; HB = Hepatoblastoma; Hep293TT = HB cell line; IHC = Immunohistochemistry; MDR = Multidrug resistance; OS = Overall survival; PCR = Polymerase chain reaction; PR = Partial response; qPCR = Quantitative real time PCR; RECIST = Response Evaluation Criteria in Solid Tumors; SD = Stable disease; SIOPEL = Société Internationale d'Oncologie Pédiatrique

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AT A GLANCE COMMENTARY

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The role of cancer stem cells (CSCs) in hepatoblastoma (HB) has not been properly addressed yet. In the present study, we assessed the prognostic and predictive values of the CSC markers (*CD133*, *CD90*, and *CD44*) in 43 HB patients by immunohistochemistry and real-time polymerase chain reaction. Protein and RNA expressions were increased in our HB patients compared with normal controls. This increase correlated significantly with disease stages, response to treatment, and reduced disease-free survival. We conclude that the CSC markers could be used as prognostic and predictive factors in HB patients.

INTRODUCTION

Hepatoblastoma (HB) is the most frequent pediatric liver cancer as it constitutes approximately 79% of all primary malignant hepatic tumors in children aged <3 years. However, a worldwide incidence rate of HB is difficult to evaluate because of important differences across various ethnicities. In Egypt, HB represented 10.24% of all hepatic malignancies according to the National Cancer Institute (NCI) registry, 2007.[1,2]

A birth weight <1000 g is usually associated with a strongly increased risk of HB, whereas a moderately increased risk is associated with younger maternal age, presumptive use of infertility treatment, maternal smoking, and higher maternal prepregnancy body mass index.[3] Moreover, 90% of HB patients have high serum α-fetoprotein (AFP) levels, which contribute to disease activity. Therefore, AFP level at diagnosis and the changes in this level during treatment should be compared with the corresponding age-adjusted values.[4] Complete surgical resection and cisplatin-containing chemotherapy are crucial for achieving cure in HB. In addition, excellent outcome was achieved in patients with stage I-un-favorable histology and stage II and in subsets of patients with stage III disease.[5,6]

Several histologic subtypes of HB are known, including the epithelial tumors, with pure fetal and mixed fetal/embryonal histology, the mixed epithelial and mesenchymal tumors, and several types of transitional, small, and large cell undifferentiated tumors. This heterogeneity reflects the distinct patterns of hepatic embryogenesis, explains the varied clinical behavior, and suggests a cancer stem cell (CSC) origin.[7,8]

The CSCs are newly identified subpopulations, which were isolated from several adult and some pediatric solid and hematologic tumors. They possess stem cell properties and can differentiate into heterogeneous progenies of malignant cells. Thus, they are probably the progenitor cells that undergo unknown genetic mutations and lose potential for tissue repair, but retain stem cell characteristics including self-renewal and plasticity to differentiate into different cell types.[8] CSCs are also responsible for tumor development, resistance to treatment, metastasis, and relapse. Therefore, a better understanding of CSC pathobiology may aid in developing novel directed therapies against these cells.[9,10]

Three major CSC markers are commonly expressed in different tumor types including *CD44*, which is a cell surface adhesion molecule mediating multiple signaling pathways. CD44 was used as a CSC marker in several tumors including breast and hepatocellular carcinoma. Other CSC markers include *CD133*, which stains the proliferative cells in multiple organs, and *CD90 (Thy-1)*, which is a glycosylphosphatidylinositol-anchored glycoprotein expressed in bone marrow–derived mesenchymal stem cell and hepatic stem cell progenitors.[11-14]

Till now, data regarding the role of CSCs in HB are still preliminary with only few studies on human samples. Using the HB cell line (Hep293TT), which was obtained from an aggressive case of HB, *CD133* overexpression was reported in HB cells and it was reduced on treatment with bortezomib and sorafenib.[15] On the other hand, Cairo et al[16] demonstrated that HB exhibits different types of stem cells, which play specific role(s) during CSC-dependent tumorigenesis. The expression levels of CSC markers efficiently categorized HB into 2 main classes: (1) the poorly differentiated types, which express fewer SC markers and (2) the well-differentiated types, which were positive for most of the SC markers. Interestingly, these markers were expressed in the neoplastic cells only, whereas the adjacent normal liver tissues were totally negative for these proteins.

Therefore, we sought to assess the possible prognostic and predictive values of the CSC markers (*CD133*, *CD90*, and *CD44*) in a well-characterized group of HB patients. This was achieved by measuring the expression levels of these markers (protein and RNA) in relation to the standard prognostic factors for HB, patients' response to treatment, and survival rates.

METHODS

Patients. The study included 43 HB patients who were recruited from the Pediatric Oncology Clinics, NCI, Cairo University, and the Children Oncology Hospital. Only cases with full clinical, radiologic, and pathologic data and ample amount of representative tumor samples