The Role of SOX-2 in the Diagnosis of Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is responsible for a large proportion of cancer deaths worldwide. HCC is frequently diagnosed after the development of clinical deterioration at which time survival is measured in months. HCC diagnosis by biomarker SOX2 which contributes of tumorigenesis and metastasis. Properties of various types of cancers strongly supported the concept that SOX2 can be used as an effective marker for HCC diagnosis. Level of expression of SOX2 was assessed with a real-time reverse transcription-polymerase chain reaction approach in a set of samples for 20 patients including serum and tissue (tumor and non-tumor) samples and the level of ALB, AST and ALT were measured. Our work reported that Expression of SOX2 in serum of normal people was down regulated than patients serum and SOX2 expression in non tumor tissue was higher than HCC tissue. It is a novel and promising biomarker for HCC diagnosis.

Results
Expression of SOX2 in tissue of normal people was higher than HCC patient on other hand Expression of SOX2 in serum of normal people was down regulated than HCC patients according to other study who examined the expression level of SOX2 in HCC tissues which has been indicated that SOX2 expression was down regulated in tissue of HCC patients and high expressed in serum of HCC patients.

Conclusion
SOX2 is discovered to be a member of a family of gene regulator, and promising biomarker for HCC diagnosis.

Introduction
Hepatocellular carcinoma (HCC) is causing of cancer in hepatic cells. HCC is a leading cause of cancer mortality, also called malignant hepatoma and is the most common type of liver cancer. Most cases of HCC are as a result of either a viral hepatitis infection (hepatitis B or C), metabolic toxins such as alcohol or aflatoxin, conditions like hemochromatosis and alpha 1-antitrypsin deficiency. (El-Serag H, 2001).[1]

Most cases of hepatocellular carcinoma occur in people who already have symptoms of chronic liver disease and cancer patients most commonly suffer from pain which is the most common and stressful symptom for them, especially in HCC patients, the abdominal pain is the most common one. This may be caused due to the visceral contribution that arises from primary or metastatic injury involving the pelvic or abdominal viscera (Mercandante, 2012).

Cancer patients including HCC patients suffer from weight loss among the terminal stage (Strasser & Bruera, 2011).[2]

There are around 625,000 new cases of HCC and almost about 650,000 HCC related to death every year in proportional with the rate of death. In Egypt, liver cancer includes the malignancies of all the digestive organs which represent about 11.60% and about 1.78% of the entire malignancies, it represents 70.50% of the liver cancers among Egyptian patients (Mokhtar, 2007).[3]

SOX2 is a gene that encodes for a transcription factor belonging to the SOX gene family and located on chromosome 3q26.3–q27, [4]it belongs to the SOXB1 group and encodes for a protein consisting of 317 amino acids contains a high-mobility group (HMG) domain,[5] which permits highly specific DNA binding. Consequently, SOX2 functions as an activator or suppressor gene transcription[6]. SOX2 has been described as an essential embryonic stem cell gene and moreover, a necessary factor for induced cellular reprogramming. (Wegner, 1999).[9] Tumor development occurs when a cell accumulates genetic alterations that modify normal cell cycle.
progression. (Sinclair, 1990). [10] There are several means of genetic aberrations including gene deletions or gene amplification. Gene amplification is defined as a copy number increase of a particular chromosomal region.[11] SOX2 amplification is due to multiplication of the 3q26.3 gene locus. (Guth, 1990).[12]

SOX2 is relevant to tumorigenicity and metastasis. (Berta P, 1990). Molecular index for the diagnosis of hepatocellular carcinoma based on SOX2 diagnostic marker whose specificity and level of expression are the most discriminating for the diagnosis of HCC. [13]

The aim of this study is to assess hepatic tissue expression of SOX2 in HCC tumor and adjacent non tumor tissues, and to know the efficacy of SOX2 utility in HCC diagnosis and to assign clinic pathological correlations.[14]

Patients & methods
This study was conducted on 20 patients who had archival tissue specimens obtained from Pathology Department, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The specimens were classified into:

Group 1: 20 tumor tissues and their corresponding non tumor tissues.

Group 2: 20 serum samples from ( HCC patients) (5 ml venous blood ) and 5 ml blood samples were taken from 10 healthy volunteers. Where there are two criterias; inclusion criteria the patients ages range from 41 to 59 years where the amount of ALT > 30 IU/L and the exclusion criteria includes ages above 60 years that has HBV, HCV, and HIV.

Methods

First RNA extraction
Tumor and non tumor tissues were homogenized , 200 µl tissue mixture was added , Subsequently RNA was extracted from tissue and 200 µl serum using Abbot RNA extraction kit m sample Preparation system Kit , cat . no .(02k02-96 ) (Abbot Molecular , Inc ,Des plaines , IL). Finally RNA was stored at -80 °C.

RNA concentration and purity was measured using Nano drop Measurement ( Nano drop 2000 c spectrophotometer ,thermo scientific ,USA). RNA concentration was between 2.1 to 18.5 ng/ µl and the purity was 1.8 to 2.

Reverse transcription:

Reverse transcription was applied on 5 µl of the extracted RNA using (Revert Aid First Strand cDNA .kit cat .k1621) that was added to 20 µl mixture solution which contains Ribo lock RNAase Inhibitor 3 µl, 5x Reaction buffer 3 µl ,oligo (dT)18 primer 1m ,water nuclease –free 5ml ,10 µl dNTP Mix 4ml, RevertAid M_Mul V RT (200U/MI) 5 ml, then samples were incubated for 60 min at 42 °C and reaction was terminated at 70 °C for 5 min.

SOX2 gene expression was determined using Maxima syber green qPCR Master mix (2x) 25 µl master mix was prepared which contains ( ROX 12.5 µl, water 4.5 µl ) 0.3mM Forward Primer 1.5 µl 5′— GCC TGG GCG CCG AGT GGA —3′ , Reverse 5′— GGG CGA GCC GTT CAT GTA GGT CGT—3′ and 5 µl cDNA , amplification was done at 95 °C for 10 min , 95 °C for 15 sec 40 cycle ,60 °C for 30 sec 40 cycle, SOX2 expression was quantified using relative quantification method ( 2 - ∆ ∆ ct).

Results

The level of albumin (ALB) in serum was higher in HCC than normal people with p=0.0001**(very high significance). A clear difference in alanine aminotransferase (ALT) level, it was higher in HCC patients than in normal people p=0.0238* (very significant). Confirming that the level of aspartate aminotransferase (AST) in serum was higher in HCC samples; with a p=0.0286* ( high significance) , tumor grade was in range of 12/22/16 and Fibrosis score (0-2)(3-4) in HCC patient 8/42 as illustrated in fig. (1).

Figures and Tables

Patient’s Clinical parameters
Table (1) ALB; albumin, ALT; alanine aminotransferase,AST; aspartate aminotransferase, NS; non-significant, *** significant at p<0.001,* significant at p<0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCC Patients</th>
<th>Healthy Volunteers</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>(50.71±9.03)</td>
<td>(41.66±8.02)</td>
<td>0.0374*</td>
</tr>
<tr>
<td>ALB g/dl</td>
<td>(2.84±0.17)</td>
<td>(4.2±0.26)</td>
<td>0.0001***</td>
</tr>
<tr>
<td>ALT IU/L</td>
<td>(48.16±3.50)</td>
<td>(26.37±1.18)</td>
<td>0.0238*</td>
</tr>
<tr>
<td>AST IU/L</td>
<td>(42.85±3.39)</td>
<td>(23.56±2.14)</td>
<td>0.0286*</td>
</tr>
<tr>
<td>Tumor grade I/II/III</td>
<td>12/22/16</td>
<td>------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Fibrosis score(0-2)</td>
<td>8/42</td>
<td>------------------</td>
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(3-4)
Figure (1): Expression of SOX2 in serum of normal people was down regulated than HCC serum, programmed at p=0.027*. The SOX2 expression RQ=2 - ∆ ∆ ct , N: normal serum ,T: HCC serum.

Figure (2): SOX2 expression in non tumor tissue was higher than HCC tissue programmed at p=0.022* (very significant). The sox2 expression RQ=2 - ∆ ∆ ct , NT: non tumor ,T: tumor tissue.

4. Discussion

Hepatocellular carcinoma is a cancer that starts in the liver. It's different from "secondary" liver cancers, which have spread to the liver from other organs. If caught early, it can sometimes be cured with surgery or transplant. In more advanced cases it can’t be cured. HCC can be detect at early stage by using SOX2 as diagnostic marker. SOX2 is a gene that encodes for a transcription factor belonging to the SOX gene family and located on chromosome 3q26.3–q27, belongs to the SOXB1 group and encodes for a protein consisting of 317 amino acids contains a high-mobility group (HMG) domain. SOX2 amplified in various cancer types and affect cancer cell physiology via involvement in complicated cell signaling and protein-protein interactions(Rossi DJ, 2006). Twenty patients were included in this study with samples of tumor and non tumor tissues, and serum samples were collected from each patient. According to our study, through comparing the level of albumin in healthy volunteers and HCC patients; the level of albumin in serum became higher in HCC than normal people with p=0.0001*** (high significant value). It indicates that there was a different level of albumin in HCC patients and the normal people. This result was in relation with previous study for (Kusakabe, 2011) which stated that the recurrence rate of HCC was low associated with high serum albumin level in patients. Concerning the level of alanine aminotransferase through a correlation used between HCC patients and healthy volunteers; a clear difference in ALT level was observed, It was higher in HCC patients than in normal people p=0.0238* (very significant). It is agreed by (Christoph E., et al., 2007) who reported that HCC patients had higher level of alanine aminotransferase than in normal people. It distinguishes between the serum aspartate aminotransferase (AST) level and the clinical parameters. Confirming that the level of aspartate aminotransferase in serum which is higher in HCC samples; with a p=0.0286* (high significance) It is agreed by (Edoardo G., et al. 2003) who reported that HCC patients had higher level of alanine aminotransferase than in normal people. Expression of SOX2 in tissue of normal people was higher than HCC patients programmed at p=0.022* (very significant) on other hand Expression of SOX2 in serum of normal people was down regulated than HCC patients ,programmed at p=0.027* , According to (Fang X., et al. 2010), (Neumann J., et al. 2010) who examined the expression level of SOX2 in HCC tissues which has been indicated that SOX2 expression was down regulated in tissue of HCC patients and high expressed in serum of HCC patients. SOX2 is discovered to be a member of a family of gene regulators. It was demonstrated that the SOX2 has a critical role in several carcinogenesis-related events, such as migration, anti-apoptosis, angiogenesis, transformation and proliferation in many types of solid tumors, including HCC’s (Muramatsu, 2013). In a conclusion SOX2 is a novel and promising biomarker for HCC diagnosis.

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5. References


[11] Lea F. MD; Frank, Renee MD; Zhang, Paul J. MD; Furth, Emma E. MDAmerican Journal of Surgical Pathology: February 2014 - Volume 38 -