The High Expressed Serum Soluble Neural Cell Adhesion Molecule, a High Risk Factor Indicating Hepatic Encephalopathy in Hepatocellular Carcinoma Patients

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Abstract

Objective: To investigate whether the expression of serum soluble neural cell adhesion molecule (sNCAM) is associated with hepatic encephalopathy (HE) in hepatocellular carcinoma (HCC) patients. Materials and Methods: The Oncomine Cancer Microarray database was used to determine the clinical relevance of NCAM expression in different kinds of human cancers. Sera from 75 HCC cases enrolled in this study were assessed for expression of sNCAM by enzyme linked immunosorbent assay (ELISA). Results: Dependent on the Oncomine Cancer Microarray database analysis, NCAM was down regulated in 10 different kinds of cancer, like bladder cancer, brain and central nervous system cancer, while up-regulated in lung cancer, uterine corpus leiomyoma and sarcoma, compared to normal groups. Puzzlingly, NCAM expression demonstrated no significant difference between normal and HCC groups. However, we found by quantitative ELISA that the level of sNCAM in sera from HCC patients with HE (347.4±151.9 ng/ml) was significantly more up-regulated than that in HCC patients without HE (260.3±104.2 ng/ml), the p-value being 0.008. sNCAM may be an important risk factor of HE in HCC patients, the correlation coefficients was 0.278 (P< 0.05) on rank correlation analysis. Conclusions: This study highlights that up-regulated level of serum sNCAM is associated with HE in HCC patients and suggests that the high expression can be used as an indicator.

Keywords: Soluble neural cell adhesion molecule - hepatic encephalopathy - hepatocellular carcinoma
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Aspartate transaminase; AFP, alpha fetoprotein; HbsAg, hepatitis B surface antigen.

| Description                        | HCC patients with HE | HCC patients without HE |
|------------------------------------|-----------------------|-------------------------|
| Number of individuals              | 46                    | 29                      |
| Gender (male/female)               | 36 (78.3%)/10 (21.7%)/| 36 (79.3%)/10 (20.7%)/  |
| Age (years)                        | 59±9                  | 59±11                   |
| ALT (IU/L)                         | 47.7±36.6/10 (21.7%)/ | 55.2±45.4/6 (20.7%)/    |
| AST (IU/L)                         | 70.9±71.2/10 (21.7%)/ | 98.3±101.9/6 (20.7%)/   |
| AFP (IU/ml)                        | 303.9±480.2/15.2%     | 439.9±548.3/17.2%       |
| HbsAg(s/co) (1/0)                  | 39 (84.8%)/7 (15.2%)/ | 24 (82.8%)/5 (17.2%)/   |
| PT(s)                              | 14.5±2.1/14.0±1.9     |

*Mean±standard deviation ALT, Alanine aminotransferase; AST, Aspartate transaminase; AFP, alpha fetoprotein; HbsAg, hepatitis B surface antigen; PT, Prothrombin time

Oncomine analysis

Oncomine Cancer Microarray database (http://www.oncomine.org/) was used to systematically assess expression levels of NCAM in various cancers tissues versus normal tissues. (Rhodes et al., 2004; Shan et al., 2015) Threshold by $P\leq0.001$, fold change $\geq2$ and gene rank was top10%. The corresponding data sources used in this study were summarized in Table2.

Enzyme linked immunosorbent assay (ELISA)

sNCAM was measured quantitatively in sera from 75 patients with HCC by using the NCAM1 (Human) ELISA Kit (Abnova), according to the manufacturer’s protocol. Briefly, the diluted sera and standards were pipetted into the detective plate and incubated at 37°C for 90 min. Then biotinylated antibodies were pipetted. After incubating and washing, Avidin-Biotin-Peroxidase Complex (ABC) working solution was added, and finally, a color development step was performed. The O.D. absorbance values were read at 450 nm using the Infinite M200 (Tecan).

Statistics

The student t-test was used to compare two groups of parametric variants. The correlation of serum sNCAM expression and HE in HCC patients was evaluated with spearman rank correlation analysis. SPSS 17.0 was used to process the statistical analysis and GraphPad prism 5.0 was used to draw the graphs. $P\leq0.05$ was considered statistically significant.

Results

NCAM expression in cancer tissues

To determine the clinical relevance of NCAM in different kinds of human cancers, NCAM expression in bladder cancer, brain and central nervous system cancer, breast cancer, cervical cancer, colorectal cancer, gastric cancer, head and neck cancer, lung cancer, lymphoma, other cancer (uterine corpus leiomyoma), ovarian cancer, prostate cancer, sarcoma and so on were from Oncomine Cancer Microarray database. We compared NCAM expression levels in cancer tissues to that in normal tissues with the threshold by $p$-value below 0.001, fold change $\geq2$, gene rank was top10%. The result was listed in Figure 1 A. It indicated that 29 analyses met all of these conditions. The results demonstrated that NCAM expression was significantly changed in 13 different cancers versus normal tissues, respectively. In bladder cancer, brain and central nervous system cancer, breast cancer, cervical cancer, colorectal cancer, gastric cancer, head and neck cancer, lymphoma, ovarian cancer and prostate cancer, the expressions of NCAM were significantly decreased. While, in lung cancer, other cancer (uterine corpus leiomyoma) and sarcoma there were significantly higher expression levels of NCAM.

However, any studies of the NCAM expression in HCC versus normal tissues were outside the scope mentioned above. Oncomine Cancer Microarray database collected 8 analyses of the NCAM expression in HCC tissues versus normal tissues. While only one of them had a $p$-value
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We studied the expression levels of serum sNCAM by ELISA. 75 sera from HCC patients including 46 sera from HCC patients with HE and 29 sera without HE. The mean level of sNCAM in the sera of HCC patients with HE and without HE was 347.4±151.9 ng/ml and 260.3±104.2 ng/ml, respectively. The expression of serum sNCAM in HCC with HE was significantly up regulated compared to the normal controls.

Table 2. Oncomine Microarray Data Were Used to Analyze the NCAM Expression in Human Cancers

| Analysis type           | Study                          | Sample type                               | Samples (n) | Year of the study | References                        |
|-------------------------|--------------------------------|-------------------------------------------|-------------|-------------------|-----------------------------------|
| Normal vs. Cancer       | Bladder Cancer                 | Infiltrating Bladder Urothelial Carcinoma | 81          | 2006              | (Sanchez-Carbayo et al., 2006)    |
|                         |                                | Superficial Bladder Cancer               | 28          |                   |                                   |
|                         |                                | Bladder                                   | 48          |                   |                                   |
| Brain and CNS Cancer    | Brain                          | Glioblastoma                               | 5           | 2013              | The Cancer Genome Atlas           |
|                         |                                | Brain                                     | 13          | 2004              | (Dyrskjot et al., 2004)           |
|                         | Accompanied Tumor              | Brain                                     | 9           |                   |                                   |
| Breast Cancer           | Invasive Ductal Breast Cancer  | Brain                                     | 35          | 2004              | (Zhao et al., 2004)               |
|                         |                                | Brain                                     | 3           |                   |                                   |
| Cervical Cancer         | Cervical Squamous Cell Cancer  | Brain                                     | 3           | 2011              | The Cancer Genome Atlas           |
|                         |                                | Brain                                     | 61          |                   |                                   |
| Colorectal Cancer       | Cecum Adenocarcinoma           | Colon                                     | 22          | 2011              | (Sanchez-Carbayo et al., 2011)   |
|                         |                                | Colon                                     | 13          |                   |                                   |
|                         | Brain                          | Brain                                     | 25          | 2007              | (Sabates-Bellver et al., 2007)    |
| Gastric Cancer          | Gastric Cancer                 | Colon                                     | 25          | 2007              | (Cui et al., 2011)                |
|                         |                                | Colon                                     | 25          |                   |                                   |
|                         | Gastric Mixed Adenocarcinoma   | Colon                                     | 25          | 2007              | (Cui et al., 2011)                |
|                         | Gastric Intestinal Type        | Colon                                     | 25          |                   |                                   |
|                         | Adenocarcinoma                 | Brain                                     | 25          |                   |                                   |
| Head and Neck Cancer    | Tongue Squamous Cell Cancer    | Tongue                                    | 26          | 2009              | (Estilo et al., 2009)             |
|                         |                                | Thyroid Gland Pallidary Carcinoma         | 9           |                   |                                   |
|                         |                                | Thyroid Gland                             | 9           |                   |                                   |
| Lung Cancer             | Lung Carcinoid Tumor           | Small Cell Lung Carcinoma                 | 6           | 2001              | (Bhattacharjee et al., 2001)      |
|                         |                                | Lung                                       | 17          |                   |                                   |
| Lymphoma                | Follicular Lymphoma            | Plasmacytoma                               | 5           | 2008              | (Brune et al., 2008)              |
|                         |                                | Plasma Cell                               | 5           |                   |                                   |
| Other Cancer            | Uterine Corpus Leiomyoma       | Small Cleaved Follicle Center Cell        | 5           | 2009              | (Crabtree et al., 2009)           |
|                         |                                | Myometrium                                | 50          |                   |                                   |
|                         |                                | Myometrium                                | 27          |                   |                                   |
| Ovarian Cancer          | Ovarian Serous Adenocarcinoma  | Peritoneum                                 | 10          | 2009              | (Yoshihara et al., 2009)          |
|                         |                                | Prostate Carcinoma                        | 25          | 2001              | (Welsh et al., 2001)              |
| Prostate Cancer         | Prostate Gland                 | Prostate Gland                            | 9           |                   |                                   |
| Sarcoma                 | Dedifferentiated Liposarcoma   | Prostate Gland                            | 46          | 2009              | (Barretina et al., 2010)          |
|                         |                                | Leiomyosarcoma                            | 26          |                   |                                   |
|                         |                                | Adipose Tissue                            | 9           |                   |                                   |

below 0.001 (Mas et al., 2009) and the fold change was only 1.3 (Figure 1 B). By contrast, there was no significant change of NCAM expression in HCC versus normal tissues. This result implied that NCAM may be not a good biomarker for HCC diagnosis. The quantitative analysis of serum sNCAM expression in HCC patients with or without HE.
Table 3. Spearman Rank Correlation Coefficients and Probabilities between Serum sNCAM Expression and HE in HCC Patients

|                       | HE                       | sNCAM                      |
|-----------------------|--------------------------|----------------------------|
| Spearman’s rho        | HE                       | sNCAM                      |
| correlation coefficients | 1.000                    | 0.278*                     |
| P value (2-tailed)     | 0.000                    | 0.016                      |
| N                     | 75                       | 75                         |

| sNCAM                  | HE                       | P value (2-tailed) |
|------------------------|--------------------------|--------------------|
| correlation coefficients | 0.278*                   | 1.000              |
| N                      | 75                       | 75                 |

*N, number; *Correlation is significant at the 0.05 level (2-tailed)

Discussion

Generally, HE was divided into three types due to its etiology and pathogenesis: type A associated with acute liver failure; type B related to portal-systemic bypass and no intrinsic hepatocellular disease; type C involved in cirrhosis and portal hyper-tension/or portal-systemic shunts (Ferenci et al., 2002). In China, most HE patients were type C, type A and type B just occupied a relatively small minority. Recent advances have fostered a further understanding of the pathogenesis of HE, but the more detailed investigation about mechanism of HE is needed and it would be crucial to improve the therapeutic effect. If HE can be detected timely and prevented properly, it may be able to reduce the incidence and mortality of HE by taking active therapeutic measures.

In this study, we found expression of serum sNCAM in HCC patients with HE was significantly upregulated than that in HCC patients without HE. There are 3 major isoforms of NCAM as follows: NCAM-180, NCAM-140, and NCAM-120, with molecular masses of 180, 140, and 120 kDa, respectively. The NCAM-120 with no intracellular residues is linked to the membrane via a glycosyl-phosphatidylinositol (GPI) anchor, while NCAM-140 and NCAM-180, have intracellular parts of different lengths (Cunningham et al., 1987). Since NCAM could be released to serum by shedding with or without transmembrane domains as a detectable soluble form of NCAM (Tsuchiya et al., 2011). It implied that using a blood test to assess the sNCAM in the serum may be a convenient method for diagnosis and monitor of HE.

However, the diagnostic value of serum sNCAM in HCC patients with HE needs to be further validated in a large scale investigation and that how sNCAM participates in HE progression also needs to be evaluated. What is more, NCAM is an important glycoprotein with six possible N-linked glycosylation sites. It can carry high levels of the negatively charged polysialic acid (PSA) which consists of a 2-8 linked N-acetyلهورانmic acid residues (Livingston et al., 1988). Whether the expression of PSA-NCAM is related to HE progression also requires to be further elucidated.

In conclusion, we report the expression level of NCAM in cancer tissues versus normal tissues according to Oncomine Cancer Microarray database and expression of serum sNCAM in HCC patients with HE was significantly up regulated compared to the expression in HCC patients without HE expression in HCC patients without HE and the p-value was 0.008. Rank correlation analysis demonstrated that positive rank correlation existed between sNCAM and HE in HCC patients, the correlation coefficients was 0.278 (P<0.05).

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