Is high AgNOR quantity in hepatocytes associated with increased risk of hepatocellular carcinoma in chronic liver disease?

M Derenzini, D Trerè, F Oliveri, E David, P Colombatto, F Bonino, M R Brunetto

Abstract

Aims—To evaluate whether high numbers of silver staining nucleolar organiser regions (AgNORs) in hepatocytes are associated with increased risk of hepatocellular carcinoma in chronic liver disease.

Methods—The quantitative distribution of AgNORs was studied in the liver biopsy specimens of 33 patients with chronic liver disease, 11 of whom developed hepatocellular carcinoma. The interval between liver biopsy and diagnosis of hepatocellular carcinoma was 26 months (range one to 61 months); the mean follow up of patients without hepatocellular carcinoma was 45 months (range 24–59 months). Quantitative evaluation of AgNORs was carried out on silver stained routine sections by morphometric analysis, using a computer assisted image analysis system.

Results—High interphase AgNOR values (>3 μm²) were found in hepatocytes of nine out of the 11 (82%) patients in whom neoplastic transformation occurred. Of the remaining 22 patients, only seven (31%) had AgNOR values higher than >3 μm² (χ² 4·83; p = 0·036).

Conclusions—These results indicate that high numbers of interphase AgNORs are associated with increased risk of hepatocellular carcinoma in patients with chronic liver disease.

(Hepatocellular carcinoma is the fourth commonest cause of death from neoplasia in the world (250 000 cases a year).1 It is frequently associated with chronic liver diseases such as cirrhosis and chronic hepatitis, and the duration of cirrhosis and necroinflammation are considered to be major risk factors. Surgical resection or medical treatment can improve survival, although patients with hepatocellular carcinoma must be diagnosed sufficiently early to benefit from these forms of treatment.1 Predictive tests are therefore mandatory for the screening of patients with chronic liver disease.

In experimental hepatocarcinogenesis intermediate non-cancerous lesions, characterised by a progressive increase of hepatocyte proliferative activity, precede the onset of cancer.2 Tanaka et al correlated the proliferative activity of hepatocellular carcinoma induced by N-2-Fluorenylacetamide with the mean number of silver stained interphase nucleolar organiser regions (AgNORs) in hepatocyte nuclei.3 Interphase NORs are nucleolar components containing ribosomal genes associated with a group of argyrophilic proteins.4,5 Evidence suggests that the numbers of interphase AgNORs are directly related to cell proliferation rate. A strict correlation was in fact observed between interphase AgNOR values and the percentage of S-phase cells, determined by either DNA flow cytometry or bromodeoxyuridine (BrdU) incorporation, in non-Hodgkin's lymphomas,6 breast tumours,7 and meningiomas.8 Interphase AgNOR numbers and cell proliferative indices, evaluated by BrdU labelling and Ki-67 immunostaining, were also found to be directly related in cancer tissues of different origin.9 A linear relation between interphase AgNOR number and rapidity of cell proliferation has also been shown.10,11

We studied the correlation between the hepatocyte proliferation rates, determined by AgNOR quantitative evaluation, and the risk of hepatocellular carcinoma in patients with chronic liver disease followed up for 15 to 74 months after the liver biopsy.

Methods

The liver biopsy specimens of 33 patients with chronic liver disease were studied retrospectively. Eleven subsequently developed hepatocellular carcinoma during follow up at the Department of Gastroenterology, Molinette University Hospital, Turin, between 1985 and 1992. The interval between the liver biopsy and diagnosis of hepatocellular carcinoma was 26 months (range one to 61 months), while the mean follow up of patients without hepatocellular carcinoma was 45 months (range 24–59 months). There was no significant difference between the two groups regarding sex, sociodemographic features, and severity of liver disease. The median age of the patients with hepatocellular carcinoma (59 years, range 23–69 years) was higher than that of patients without hepatocellular carcinoma (43·5 years, range 28–64 years).

The level of intrahepatic inflammation was determined by the method of Knodell and coworkers.12

Cell kinetic analysis was performed by measuring the interphase AgNOR numbers on routine histological sections of 33 liver biopsy specimens stained by the silver method according to Ploton and coworkers.13

Centre for Cellular Pathology, Department of Experimental Pathology, University of Bologna, Italy
M Derenzini D Trerè
Department of Gastroenterology, Ospedale Molinette, Turin, Italy
F Oliveri P Colombatto F Bonino M R Brunetto
Department of Biomedical Sciences and Human Oncology, University of Turin, Italy
E David

Correspondence to: Professor Massimo Derenzini, Centro di Patologia Cellulare, Dipartimento di Patologia Sperimentale, University of Bologna, Via San Giacomo 14, 40126 Bologna, Italy.
Accepted for publication 23 February 1993

http://jcp.bmj.com/ on June 22, 2017 - Published by group.bmj.com
higher than those (mean value 3.00 (0.91) 
\mu m^2, range 2-01 to 5-02 \mu m^2) in patients
without hepatocellular carcinoma (Fisher's
exact test: (univariate) odds ratio 7.9; 95%
certainty interval 1.4-45-9; p = 0.0255).

We identified two groups of patients
according to their mean AgNOR values,
using a cutoff value of 3 \mu m^2 that had already
been established as borderline between
rapidly and slowly proliferating tissues.10
Seventeen cases (group 1) had a mean
AgNOR value smaller than 3 \mu m^2 and
the other 16 (group 2) greater than 3 \mu m^2. Two
patients out of 17 (11-8%) of group 1 devel-
opated hepatocellular carcinoma compared
with nine patients out of 16 (56-2%) in group
2 (x^2 4.83; p = 0.036).

Figures 1 and 2 show two cases with a
different AgNOR number. One patient in
figure 1 (case 5) with a low AgNOR value
showed no evidence of hepatocellular carci-
noma 52 months after the diagnosis of cirrho-
sis (fig 1). One case in group 2 (case 30) with
a high AgNOR value developed the disease
20 months after cirrhosis had been diagnosed
(fig 2).

Variations in AgNOR values were not sig-
nificantly associated with a different preva-
ence of cirrhosis and inflammation in the two
groups. Cirrhosis was present in nine of 17
patients in group 1 patients and in 10 of 16 group 2
patients; severe inflammation occurred in 13
of 17 and in eight of 16 patients, respectively.
Patients with multiple hepatitis infections
two in group 1 and eight in group 2) had
higher AgNOR values than patients with
single virus infection (14 in group 1 and six in
group 2), but this difference was not signi-
cant because of the low number of biopsy
specimens. Six of seven patients with hepatic-
itis D virus infection (three of which devel-
oped hepatocellular carcinoma) had AgNOR
values higher than 3 \mu m^2.

All the specimens had been fixed in buffered
formalin. NOR silver staining was carried out
using a solution of one volume 2% gelatine in
1% aqueous formic acid and two volumes of
50% silver nitrate. The staining reaction was
performed for 14 minutes at 37°C.

The area occupied by the interphase
AgNORs within nuclei of 100 cells a patient
was measured using a specific program
(IM 5200) of a computer assisted image
analysis system (Sistema MONO, Immagini e
Computer, Milan).

Statistical analysis of measured data was
performed using the \chi^2 test and Fisher's
exact test. Statistical significance was defined at
p < 0.05.

Results
The mean AgNOR areas of the 33 cases eval-
uated ranged from 2-01 to 7-42 \mu m^2 (table). AgNOR
values in the liver biopsy specimens
of patients who developed hepatocellular
carcinoma (mean (SD) value 4-48 (1-49) \mu m^2,
range 2-55 to 7-42 \mu m^2) were significantly

Discussion
It is well known that the quantity of inter-
phase AgNORs is greater in neoplastic than in
corresponding benign or normal tissues.14 16
As far as liver pathology is concerned,
Crocker and McGovern showed that hepato-
cellular carcinoma was always characterised
by a greater quantity of interphase AgNORs
than cirrhotic liver.17 In the same study the
authors observed that interphase AgNOR
numbers in dysplastic cirrhosis fell between
those in cirrhosis alone and hepatocellular
carcinoma. The quantity of interphase
AgNORs is strictly related to cell proliferative
activity.15 16 AgNOR value increases during G_1
phase to reach its peak during S-phase.18 In
proliferating cells the faster the cell prolife-
ration the greater the interphase AgNOR
activity.12 The results of our study indicate that a
high hepatocyte proliferation rate, as mea-
sured by high AgNOR numbers in the hepato-
cyte nuclei of patients with chronic liver
disease, is associated with a higher incidence
of hepatocellular carcinoma: 82% of patients
who developed cancer had an AgNOR value
greater than 3 μm², compared with only 31% without hepatocellular carcinoma. The proliferation rates observed in patients with multiple hepatitis infections (in particular with hepatitis D superinfection) and with chronic liver lesions are higher than those of patients with just one virus infection, suggesting that hepatitis viruses may have a synergistic role in the deregulation of cell proliferation. These findings are consistent with epidemiological, clinical, and pathological data, indicating that chronic hepatitis D virus infection in patients with chronic liver disease is associated with the development of hepatocellular carcinoma at an early age.19,20 This hypothesis prompts further study on a larger number of cases.

In conclusion, AgNOR quantitative analysis can be used to evaluate the hepatocyte proliferation rate in routine sections from small needle biopsy specimens. A cutoff value of 3 μm² can represent a valuable parameter to identify patients at high risk of developing hepatocellular carcinoma. Its predictive value has to be confirmed in a larger series of patients.

This work was supported by Grants from MURST (40% and 60%), Pallottinis Legacy for Cancer Research, and Regione Emilia-Romagna (DGR 4243/1991). Part of this study was sponsored by the CNR Contract No 92.02300.PF59.


AgNORs and development of hepatocellular carcinoma

Clinical features of patients and results of AgNOR area detection

<table>
<thead>
<tr>
<th>Case No</th>
<th>Ag at biopsy</th>
<th>Antimicrobial of liver disease</th>
<th>Histology</th>
<th>Hepatocellular carcinoma</th>
<th>AgNOR area (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>HCV</td>
<td>CAH A/cirrhosis/steatosis</td>
<td>No</td>
<td>2-01 (0.50)</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>HBV</td>
<td>CAH B</td>
<td>No</td>
<td>2-06 (0.54)</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>HCV</td>
<td>CAH B</td>
<td>No</td>
<td>2-12 (0.70)</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>HBV</td>
<td>CAH A/cirrhosis</td>
<td>No</td>
<td>2-21 (0.77)</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>HBV</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-29 (0.73)</td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>HBV</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-31 (0.74)</td>
</tr>
<tr>
<td>7</td>
<td>59</td>
<td>NANB</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-32 (0.73)</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>HBV</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-39 (0.73)</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>HBV</td>
<td>CAH A/cirrhosis</td>
<td>No</td>
<td>2-47 (0.77)</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>HBV/HDV</td>
<td>CAH A/cirrhosis</td>
<td>No</td>
<td>2-51 (0.80)</td>
</tr>
<tr>
<td>11</td>
<td>66</td>
<td>Haemocromatosis</td>
<td>CAH A/siderosis</td>
<td>Yes</td>
<td>2-55 (0.47)</td>
</tr>
<tr>
<td>12</td>
<td>66</td>
<td>HCV</td>
<td>CAH B</td>
<td>Yes</td>
<td>2-59 (0.65)</td>
</tr>
<tr>
<td>13</td>
<td>53</td>
<td>HBV</td>
<td>CAH A/cirrhosis</td>
<td>No</td>
<td>2-77 (0.90)</td>
</tr>
<tr>
<td>14</td>
<td>55</td>
<td>HBV</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-85 (0.92)</td>
</tr>
<tr>
<td>15</td>
<td>36</td>
<td>HBV</td>
<td>CAH A/cirrhosis</td>
<td>No</td>
<td>2-90 (0.77)</td>
</tr>
<tr>
<td>16</td>
<td>39</td>
<td>HBV/HDV</td>
<td>CAH B</td>
<td>No</td>
<td>2-94 (0.79)</td>
</tr>
<tr>
<td>17</td>
<td>58</td>
<td>HBV</td>
<td>CAH B/cirrhosis</td>
<td>No</td>
<td>2-96 (0.79)</td>
</tr>
</tbody>
</table>

CAH: A chronic active hepatitis with mild or moderate activity; CAH B: chronic active hepatitis with severe activity; HBV hepatitis B virus; HCV hepatitis C virus; HDV hepatitis D virus; NANB non-A, non-B hepatitis.
Is high AgNOR quantity in hepatocytes associated with increased risk of hepatocellular carcinoma in chronic liver disease?

M Derenzini, D Trerè, F Oliveri, E David, P Colombatto, F Bonino and M R Brunetto

doi: 10.1136/jcp.46.8.727

Updated information and services can be found at:
http://jcp.bmj.com/content/46/8/727

**Email alerting service**

These include:

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/