Available online at www.sciencedirect.com
ScienceDirect
Journal of Clinical Neuroscience 14 (2007) 355–358
Journal of Clinical Neuroscience
www.elsevier.com/locate/jocn

Laboratory study

# Significance of cyclin D1 expression in meningiomas: A preliminary study

Angela Alama [a,*], Federica Barbieri [a], Renato Spaziante [b], Cristina Bruzzo [a], Patrizia Dadati [c], Alessandra Dorcaratto [d], Jean Louis Ravetti [c]

[a] *Experimental Pharmacology, National Institute for Cancer Research, Largo Rosanna Benzi, 10, 16132 Genova, Italy*
[b] *Department of Neuroscience, Ophthalmology and Genetics, Division of Neurosurgery University of Genova, Genova, Italy*
[c] *Section of Pathology, San Martino Hospital, Genova, Italy*
[d] *DISCAT Section of Neurosurgery, Genova, Italy*

Received 30 November 2005; accepted 4 April 2006

**Abstract**

Forty-four evaluable patients with intracranial meningiomas were assessed for the expression of the cell-cycle regulator cyclin D1 and of proteins involved in proliferation and apoptosis such as PCNA, MIB-1, p53 and bcl-2. Analyses were carried out by western blot and immunohistochemistry after immediate processing of fresh tumor specimens. By western blot, expression of cyclin D1 significantly correlated with p53 ($p = 0.02$) and with proliferative activity, as assessed by PCNA expression ($p = 0.0009$). By immunohistochemistry, a significant relationship between cyclin D1 and the proliferation marker MIB-1 was confirmed ($p = 0.05$), whereas significance with bcl-2 expression was not found ($p = 0.01$). Moreover, although the association with tumor grade appeared of borderline statistical significance ($p = 0.07$), all the grade II/III meningiomas showed increased expression of cyclin D1 and high proliferative activity. In conclusion, data from this preliminary study seem to suggest a potential value of the combined expression of cyclin D1 and proliferation indicators in defining subgroups of meningiomas with a more aggressive biological behavior.


*Keywords:* Intracranial meningioma; Cyclin D1; Proliferation markers

## 1. Introduction

The most common primary intracranial tumors arise from meningothelial cells and from neuroepithelial tissue.[1] The World Health Organization (WHO) classifies these neoplasms into different categories according to cell of origin and takes into account histogenesis, cellular and nuclear pleomorphisms, mitoses, endothelial proliferation and necrosis.[2]

The majority of meningiomas, which constitute between 13% and 26% of intracranial neoplasms, are benign (WHO grade I).[3]

Some subtypes, endowed with increased proliferative activity and more aggressive behavior, present biological and clinical features more consistent with transformed phenotypes and are graded into WHO grades II and III.[4,5]

In general, cell proliferation shows an increase from benign to atypical to anaplastic meningiomas as demonstrated by MIB-1/Ki-67 indices whose values of labeling above 5–10% are correlated with a higher likelihood of recurrence.[6,7] Other biological parameters, such as apoptosis and cell cycle-related factors have been examined in a number of studies but to date their predictive values have been poorly demonstrated.[8,9] During the past decades, significant advances in understanding the biological mechanisms involved in brain tumor development have been made.[10] These include the identification of pathways and molecular effectors associated with cell cycle control and apoptosis such as components of the p53/MDM2/p21 pathway and the p16/CDK4/cyclinD1/Rb pathway, regulating the progression from G1 to S phase of the cell

* Corresponding author. Tel./fax: +39 010 5737318.
*E-mail address:* angela.alama@istge.it (A. Alama).

0967-5868/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jocn.2006.04.001

cycle.[11,12] Up-regulation of cyclin D1 occurs in a number of human cancers, likely contributing to the early development of malignancy.[13] In meningiomas, the expression of apoptotic and proliferation factors such as P53 (tumor suppressor gene), BCL-2 (anti-apoptotic gene) and proliferating cell nuclear antigen (PCNA) have been demonstrated to be helpful in determining the grade of malignancy and disease recurrence.[6–8] but, as far as we know, the expression of cyclin D1 has been poorly investigated and correlations with clinico-pathological parameters are presently lacking.

To elucidate the importance of expression of genes involved in apoptosis and proliferation in meningiomas, 44 consecutive patients were entered this study. Cyclin D1, p53 and PCNA protein contents were determined by western blot on fresh tumor tissue obtained at the time of biopsy or primary surgery, while the expression of bcl-2 and MIB-1 were evaluated by immunohistochemistry.

The clinical and biological significance of cyclin D1 expression in this group of intracranial meningiomas is presented and discussed.

## 2. Patients and methods

### 2.1. Patients and surgical specimens

A total of 44 evaluable patients with meningiomas entered this study. Biopsy or surgical tumor samples were collected from the Division of Neurosurgery, University of Genova, Genova, Italy.

Thirty-two patients were female and 12 male and their clinico-pathological features are reported in Table 1.

After histological examination by the pathologist (JLR), part of the tumor tissue was mechanically disaggregated to obtain a gross cell suspension, and immediately processed for protein extraction. Histology and tumor grading were carried out at the Department of Pathology, S. Martino Hospital, Genova, according to the WHO classification[3,4] following standard diagnostic criteria.

### 2.2. Western blot analysis

Cell suspensions were washed twice in phosphate-buffered saline and dissolved in lysis buffer as previously described.[14]

The protein concentration of cell lysates was determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions.

Forty micrograms of total proteins were separated on a 12% polyacrilamide gel and then transferred onto a nitrocellulose membrane (Hybond C-Extra; Amersham Italia Srl, Milan, Italy). Blots were probed with the appropriate antibody at a dilution of 1:1000 according to the procedure previously described.[14] The anti-cyclin D1 (R-124), p53 (DO-1) and PCNA (PC-10) monoclonal antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. In order to ascertain equal loading, anti-α tubulin, diluted 1:8000 was used (Sigma-Aldrich, St Louis, MO, USA). Immunodetection was performed using the enhanced chemiluminescence (ECL) kit (Amersham Italia) following the supplier's recommended procedures.

Quantitative determination of the protein was assessed by densitometric scanning of the band from film using an Ultrascan XL densitometer (Pharmacia, Uppsala, Sweden) as previously described.[15]

### 2.3. Immunohistochemistry

Immunohistochemical detection of the biomarkers bcl-2 and MIB-1 was performed on paraffin-embedded 4 μm tissue sections stained with the following mouse monoclonal antibodies (all purchased from Menarini, Italy): anti-bcl-2 (124) diluted 1:50 and anti-MIB-1 (Ki-67) undiluted. All antibodies were applied to pre-treated sections for 1 h. Immunostaining was then performed with a biotinylated horseradish peroxidase/avidin complex (Menarini). MIB-1 positive-stained cells were evaluated by visual inspection and a Labelling Index (LI) was calculated as a percentage of positive cells/number of cells counted (at least 1000) in randomly selected microscopic fields. Bcl-2 immunopositivity was determined by scoring positive cells per representative visual field. The nuclear counterstaining was done with hematoxylin; all the slides were blindly reviewed and scored by neuropathologists (JLR, PD and AD).

Negative controls were included in all the immunohistochemical analyses by omitting the primary antibodies. Non-neoplastic cells were excluded from the counting.

### 2.4. Statistical analysis

The association between clinico-biological variables and cyclin D1 was investigated by the Kruskal-Wallis test, for two or more groups, and tested for significance according to the $\chi^2$ test ($p \leqslant 0.05$).

Table 1
Clinico-pathological features of patients

| Tumor type | Overall | Median age in years (range) | Gender | | Histology | | WHO grade |
|---|---|---|---|---|---|---|---|
| Meningioma | 44 | 67 (41–82) | Female | 32 | Meningothelial | 25 | I |
| | | | | | Fibrous | 9 | I |
| | | | Male | 12 | Transitional | 6 | I |
| | | | | | Atypical | 3 | II |
| | | | | | Anaplastic | 1 | III |