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Immunohistochemical Analysis of c-Fos and c-Jun in Retinoblastoma

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Abstract. The c-*fos* promoter is negatively regulated by the retinoblastoma (Rb)-sucept-ibility-gene- encoded protein as well as by other genes involved in the control of transcription, cell cycle regulation and neoplastic transformation. We have examined by immunohistochemistry the c-Fos and c-Jun proteins in five cases of retinoblastoma in order to evaluate eventual alterations in their expression in vivo, possibly related to a gene mutation or to loss of Rb negative control.

Key words: Retinoblastoma, Human, Immunohistochemistry, C-Fos, C-Jun

INTRODUCTION

Inactivation of the retinoblastoma gene RB has been associated with the aetiology of many types of human cancers, leading to the classification of RB as an anti-oncogene [1-3]. RB promotes cell growth arrest at the G_1-S phase of the cell cycle [4] and the introduction of the RB gene into RB-negative cell lines results in suppression of growth and tumorigenicity in vivo [5, 6].

The product of the RB gene is a ubiquitously expressed nuclear phosphoprotein with non-specific affinity for DNA [7]. Given these attributes, a role for the RB protein in regulating gene expression was suggested. Indeed, five cellular genes have been identified as targets of transcriptional regulation by RB [8]. Robbins et al. [9] demonstrated that the c-*fos* promoter was negatively regulated in transient transfection assay. *Fos* as well as *jun* was originally identified as a viral oncogene [10]. The cellular counterparts, c-*fos* and c-*jun*, code for nuclear proteins that exert multiple effects in different biological systems. Importantly, the rapid and transient induction of c-*fos* following cell stimulation by different external signals has established it as a key member of the early proto-

oncogene family and has implicated *fos* in signal transduction and control of cell proliferation [11].

The function of the c-*fos* gene product, a 55-kD nuclear phosphoprotein, has been the subject of intensive investigation. It has now been demonstrated that Fos acts as a transcriptional regulator whose function depends upon the formation of heterodimeric complexes with members of the Jun family of proto-oncogenes [12].

We have examined c-Fos and c-Jun protein expression in retinoblastoma tumor cells in order to assess a possible effect related to the lack of RB gene transcriptional control in vivo.

MATERIALS AND METHODS

Retinoblastoma specimens from 5 enucleated eyes were fixed in formalin and embedded in paraffin. 4-μm sections on organosilane- (Sigma, St. Louis Mo., USA). coated slides were deparaffinized and rehydrated with distilled water. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min at 37 °C in a moist chamber. Slides were then washed in phospate-buffered saline solution (PBS) for 5 min.

1% normal rabbit serum was applied to the sections for 30 min room temperature at (RT). The primary antibodies, mouse anti-c-FOS and mouse anti-c-JUN (Oncogene Science, Manhasset, N.Y.,USA) were diluted 1:50 in PBS. The sections were incubated overnight at 4 °C.

The avidin-biotin peroxidase complex method was used as a detection system. Incubation with the biotinylated goat anti-mouse immunoglobulin (Dako, Denmark) was followed by incubation with the avidin-biotin peroxidase complex for 30 min at RT. The sections were incubated with 0.02% diaminobenzidine in PBS and 0.075% H_2O_2: positive staining was identified by the presence of brown reaction products. All incubations were performed in a moist chamber. Negative controls were carried out by replacing the primary antibody with PBS. Sections were lightly counterstained with haematoxylin.

RESULTS AND DISCUSSION

Inactivation of both copies of the RB gene is an invariant feature of sporadic and familial retinoblastomas. The mechanisms of cell cycle negative control and tumour suppression of the RB gene are now thought to depend, at least in part, on the transcriptional regulation of cellular genes and binding to transcription factors exerted by the RB protein and several RB-related proteins [8].

The transcriptional negative control of RB on genes such as c-*myc* and c-*fos* [9, 13], coding for transcription factors and with tumorigenic potential, might help to explain the onset of a malignant phenotype. We have therefore examined by immunohistochemical analysis, the c-Fos protein in human retinoblastoma cases and also c-Jun, since the activation of transcription induced by c-Fos is necessarily mediated by interaction with AP-1, a potent, sequence-specific trans-activator of gene expression coded by the c-*jun* gene [12].

We failed to reveal any alteration in the expression of the two proto-oncogenes. Therefore, we can exclude in our cases an overexpression that might have depended either on c-*jun* and c-*fos* gene mutation or on a permanent transcription deregulation related to the absence of RB negative control.

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