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Original contribution

Replication protein A: a reliable biologic marker of prognostic and therapeutic value in human astrocytic tumors

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Summary Replication protein A is a single-stranded DNA–binding protein that is required for the stabilization of single-stranded DNA and identified in replication foci where members of cyclin-dependent kinases–cyclin complexes are also present. In this study, we investigated the expression of replication protein A1 and replication protein A2 subunits of replication protein A protein in correlation with cyclins D2 and D3 and nuclear factor κB expression and assessed their prognostic significance in 66 patients with astrocytomas. Statistically significant positive associations emerged between (*a*) replication protein A1 and replication protein A2 protein expression ($P < .0001$); (*b*) cyclins D2 and D3 expression ($P < .0001$); (*c*) replication protein A1, replication protein A2, and cyclins D2 and D3 expression and histologic grade ($P = .0001$ in all correlations); (*d*) replication protein A1 and cyclin D2 or D3 expression ($P < .0001$ in both relationships); and (*e*) replication protein A2 and cyclin D2 or D3 expression ($P < .0001$ in both relationships). Nuclear factor κB1/p50 expression was positively correlated with replication protein A1, replication protein A2, and cyclins D2 and D3 expression, although these relationships failed to retain statistical significance when they were adjusted for histologic grade. Replication protein A2 expression seemed to independently affect survival in grade IV ($P = .005$) as well as in the entire cohort ($P = .006$). None of the molecules under study seemed to influence survival in lower grades (II/III), either by univariate or by multivariate analysis. In conclusion, replication protein A1, replication protein A2, and cyclins D2 and D3 seem to have a parallel role in the promotion of cell cycle in astrocytic tumors being implicated in the malignant progression of these neoplasms. Moreover, replication protein A2 protein seems to be a useful prognostic indicator in patients with astrocytomas.

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1. Introduction

Replication protein A (RPA) is a single-stranded DNA (ssDNA)–binding protein that is essential in various processes of DNA metabolism, namely, in DNA replication, recombination, DNA damage checkpoints, and all major types of DNA repair machinery. It is a heterotrimeric complex that consists of 3 subunits termed *RPA1* (70 kd), *RPA2* (32 kd), and *RPA3* (14 kd) containing characteristic oligosaccharide/oligonucleotide-binding fold domains (OB-folds). RPA contains 6 OB-folds, each of which consists of 5 β-strands arranged in a β-barrel, a structure common among ssDNA-binding proteins [1]. There are 4 domains inside the RPA1 subunit denoted as DNA-binding domain (DBD) A, DBD-B, DBD-C, and DBD-F, whereas the other 2 subunits include only 1 such domain, named DBD-D for RPA2 and DBD-E for RPA3, respectively. However, only RPA1 and RPA2 directly bind to ssDNA by using 4 of the 6 described OB-folds (DBD-A, DBD-B, DBD-C, and DBD-D) in a sequential manner [2]. In contrast to the well-known ssDNA-binding properties of the 2 longer RPA subunits, the molecular functions of the smallest RPA3 are largely unknown. It seems, however, that it is needed for the survival of many types of DNA damage that stall or collapse replication folks [3].

Recent investigations have focused on the role of RPA1 and RPA2 phosphorylation for the accomplishment of cellular DNA replication and repair. It has been shown that the N-terminus of RPA2 is phosphorylated by cyclin-dependent kinase (CDK) 2 family of kinases during the S and G_2/M phases of the cell cycle [4-8]. Ionizing radiation induces hyperphosphorylation of RPA2 in a glioblastoma cell line (MO59J), probably by the product of ataxia-telangiectasia gene [9]. Furthermore, it was shown that RPA2 hyperphosphorylation in response to mitotic DNA damage facilitates cell entrance into G_1 phase and increases cell viability [9,10].

RPA is localized in the nucleus and is identified in the replication foci where members of CDKs are also present. The latter cooperate harmonically with cyclins and a variety of CDK inhibitors, thus ensuring an orderly progression through cell cycle. D-type cyclins comprise 3 subtypes, namely, D1, D2, and D3, and act in conjunction with CDK4 and CDK6 to promote progression through the G_1/S restriction point. Cyclin D1 is the most investigated cyclin in various tumors, including astrocytomas, and has been established as a protooncogene because both its amplification and overexpression are associated with uncontrolled cell growth leading further to oncogenesis in different tissues [11]. More than a decade ago, Sallinen et al [12] proposed up-regulation of cyclin D1, in the absence of gene amplification, as a marker of aggressive behavior in diffusely infiltrating astrocytomas. Much less is known about cyclins D2 and D3. For example, cyclin D2 amplification and overexpression have been reported in some colorectal carcinoma cell lines [13], whereas recently, Kuchiki et al [14] have shown amplification and overexpression of the CCND3 gene in a glioma cell line (CCF-STTG1).

The essential involvement of RPA1 and RPA2 in DNA replication and damage responses and the unequivocal contribution of cyclin D in cell cycle control render them potential contributors in the pathogenesis of human malignancies because the process of carcinogenesis and cancer growth depends both on the replication of genomes and the use of DNA reparatory mechanisms, which contribute to their resistance to chemotherapeutic drugs.

The aims of the present study were to investigate the immunohistochemical expression of RPA1, RPA2, cyclin D2, and cyclin D3 in 66 diffusely infiltrating astrocytomas and to identify further any potential relationship of these particular proteins to nuclear factor κB (NFκB) 1/p50. Moreover, the expression patterns of these molecules were analyzed with respect to conventional clinicopathologic parameters and patients' survival to assess any potential significance.

2. Materials and methods

2.1. Patients

This is a study of 66 patients with diffusely infiltrating astrocytomas for whom archival primary tumor material at diagnosis, before radiotherapy, was available. Patients had been diagnosed in the First Department of Pathology, Laiko Hospital, National and Kapodistrian University of Athens, and treated as well as followed up in Evangelismos and Asklepeion hospitals between 1999 and 2006. The study was approved by the ethical committee of the University of Athens Medical School, and informed consent was obtained from each patient before their enrolment in the study. The mean age of the patients was 54.5 ± 19.24 years, ranging from 19 to 84 years, whereas the male-to-female ratio was 40:26. All cases were reviewed by 3 experienced pathologists (P.K., D.K., and G.L.) and assigned a histologic grade according to the principles laid down in the latest World Health Organization (WHO) Classification [15]. There were 18 grade II, 13 grade III, and 35 (primary) grade IV astrocytomas.

During the observation period, 40 disease-specific deaths were recorded after a median period of 8.5 months (range, 2-41 months), whereas the remaining 26 patients had been followed for 6 to 104 months (median, 23 months). Treatment consisted of surgery (partial [29 patients] or near-complete [37 patients] resection) and postoperative radiation (47 patients). All patients with partially resected tumors (29) and 18 patients with near-completely excised tumors received postoperative radiotherapy (a total dose of 60 Gy in 30-33 fractions). According to the existing protocols between 1999 and 2006, no chemotherapy was administered in these tumors.