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The p53 Codon 72 Pro/Pro Genotype Identifies Poor-Prognosis Neuroblastoma Patients: Correlation with Reduced Apoptosis and Enhanced Senescence by the p53-72P Isoform[1,2]

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Abbreviations: 4-HT, 4-hydroxy-tamoxifen; ER, estrogen receptor; IR, ionizing radiation; MDM2, murine double minute; MYCN, v-*myc* myelocytomatosis viral-related oncogene neuroblastoma derived; NB, neuroblastoma; SNP, single nucleotide polymorphism

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[2]This article refers to supplementary materials, which are designated by Tables W1 and W2 and Figures W1 to W5 and are available online at www.neoplasia.com.

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Abstract

The p53 gene is rarely mutated in neuroblastoma, but codon 72 polymorphism that modulates its proapoptotic activity might influence cancer risk and clinical outcome. We investigated whether this polymorphism affects neuroblastoma risk and disease outcome and assessed the biologic effects of the p53-72R and p53-72P isoforms in p53-null cells. Comparison of 288 healthy subjects and 286 neuroblastoma patients revealed that the p53-72 polymorphism had no significant impact on the risk of developing neuroblastoma; however, patients with the Pro/Pro genotype had a shorter survival than those with the Arg/Arg or the Arg/Pro genotypes even in the stage 3 and 4 subgroup without MYCN amplification. By Cox regression analysis, the p53 Pro/Pro genotype seems to be an independent marker of poor prognosis (hazard ratio = 2.74; 95% confidence interval = 1.14-6.55, $P = .014$) together with clinical stage, MYCN status, and age at diagnosis. *In vitro*, p53-72P was less effective than p53-72R in inducing apoptosis and inhibiting survival of p53-null LAN-1 cells treated with etoposide, topotecan, or ionizing radiation but not taxol. By contrast, p53-72P was more effective in promoting p21-dependent accelerated senescence, alone or in the presence of etoposide. Thus, the p53-72 Pro/Pro genotype might be a marker of poor outcome independent of MYCN amplification, possibly improving risk stratification. Moreover, the lower apoptosis and the enhanced accelerated senescence by the p53-72P isoform in response to DNA damage suggest that patients with neuroblastoma with the p53-72 Pro/Pro genotype may benefit from therapeutic protocols that do not rely only on cytotoxic drugs that function, in part, through p53 activation.

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Introduction

Neuroblastoma (NB) is a childhood solid tumor that accounts for 8% to 10% of all childhood cancers and for ~15% of all deaths because of pediatric malignancies [1]. NB arises from neuroectodermal precursor cells of the neural crest, and therefore, tumors can develop anywhere in the sympathetic nervous system [1]. Clinically, NB is remarkably heterogeneous: age at diagnosis [2], clinical stage (based on the International Neuroblastoma Staging System [3]), and tumor histology [4] are the most important variables for predicting disease risk and selecting appropriate therapeutic protocols. Children older than 18 months, with tumor at advanced stage (3 or 4) or with unfavorable histologic findings have an adverse outcome despite intensive multimodal treatments such as high-dose myeloablative chemotherapy followed by rescue with autologous bone marrow [5]. Clinical heterogeneity correlates with several genetic abnormalities whose detection in tumor cells has further improved risk stratification [5]. V-*myc* myelocytomatosis viral-related oncogene NB-derived (MYCN) oncogene amplification [6], hemizygous deletions of chromosomal region 1p36 [7], and unbalanced gain of 17q regions [8] are the most common genomic aberrations in NB. *MYCN* amplification occurs in ~20% of cases and represents the most powerful marker of poor outcome [9]. In contrast to other malignancies, only 2% to 3% of NBs have mutations of the *p53* gene [10]. *p53* is a tumor suppressor gene that encodes for a nuclear phosphoprotein, which, on activation, regulates many biologic processes such as cell cycle checkpoints, apoptosis, and cellular senescence [11]. Impairment of such processes has important implications for clinical behavior and response to therapy of tumors with nonfunctional p53 [12]. *p53* also activates the transcription of the murine double minute (*MDM2*) gene that encodes for the major negative regulator of p53 [13]. In recent years, there has been an increasing interest in identifying and assessing the frequency of gene variants (polymorphisms) as a tool to predict interindividual cancer risk and response to cancer therapies [14].

A polymorphism is defined as a DNA sequence change that occurs in a significant proportion (>1%) of a large population [15]. The most common type of genetic variation is a single nucleotide polymorphism (SNP). For the *p53* gene, an SNP has been identified at codon 72 within exon 4 causing an Arg>Pro substitution [16]. The p53-72R isoform seems to be more potent than the p53-72P isoform in inducing apoptosis, and increased mitochondrial localization and reduced affinity of the p53-72R isoform for the p53 inhibitor iASPP are among the proposed mechanisms that may be responsible for such an effect [17,18].

In this study, we compared the frequency of the p53 codon 72 Arg/Arg, Arg/Pro, and Pro/Pro genotypes in 288 control subjects and 286 newly diagnosed NBs and correlated these frequencies with clinical-biologic variables such as age at diagnosis, primary tumor site, clinical stage, and *MYCN* amplification. We report here that patients with the Pro/Pro genotype, including those with normal *MYCN* status and advanced disease stages, seem to have a shorter 5-year survival than those with the Arg/Pro or Arg/Arg genotype.

The more aggressive disease of patients with NB with the Pro/Pro genotype correlated with lower apoptosis and enhanced survival of cytotoxic drug or ionizing radiation (IR)–treated p53-null LAN-1 cells expressing the p53-72P compared with the p53-72R isoform. By contrast, expression of the p53-72P isoform, alone or in the presence of a low concentration of etoposide, induced an increase in senescent cells.

Together, these findings suggest that, although relatively rare, the p53 codon 72 Pro/Pro genotype might identify a subgroup of patients with NB with aggressive disease independently of the status of other markers predictive of poor outcome. Moreover, patients with this genotype may respond less efficiently to treatments that induce DNA