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Jochen A. Werner · Stefan Gottschlich
Benedikt J. Folz · Tibor Goeroegh
Burkard M. Lippert · Jan-D. Maass · Heinrich Rudert

p53 serum antibodies as prognostic indicator in head and neck cancer

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Abstract p53 antibodies are a new serological parameter of unknown potential in patients with malignancies. Their occurrence has been described in various types of cancer patients. The mechanism underlying the immunization process is still unclear. We investigated the incidence of p53 serum antibodies in 143 head and neck cancer patients with an enzyme-linked immunosorbent assay. The post-therapy course of two matched study groups (n = 38 each), one p53-antibody-seropositive and one p53-antibody-seronegative, was followed up for 24 months. Thirty-nine head and neck cancer patients (27.3%) were seropositive for p53 antibodies. During the follow-up, the p53-antibody-seropositive patients accounted for more local tumor recurrences (n = 12 versus n = 8) and more tumor-related deaths (n = 11 versus n = 5) than did seronegative patients, and second primary tumors (n = 9 versus n = 0) occurred exclusively in seropositive patients. In total, therapy failures (recurrences, tumor-related deaths, second primaries) were observed in 17/38 cases (44.7%) in the p53-antibody-seropositive group and in 8/38 cases (21.1%) in the p53-antibody-seronegative group. These results, after a follow-up of 2 years, seem to indicate a prognostic value of p53 serum antibodies for therapy failure in patients with head and neck cancer.

Key words p53 serum antibodies · Prognostic indicator · Head and neck cancer

Introduction

The overall survival rate of patients with squamous cell carcinoma of the head and neck is still very poor although treatment modalities, like surgery, radiotherapy, and chemotherapy have been optimized over the last decades. The limiting factors of survival are local or regional recurrences and second primary tumors that develop in 5%–25% of patients with head and neck cancer [5]. Over the last few years many biological markers have been postulated and promoted as prognostic factors for head and neck cancer, but none has been involved in an easy-to-perform clinical routine procedure with prognostic significance for these patients.

Mutations of the p53 tumor-suppressor gene are some of the commonest genetic alterations in human cancer with a prevalence in different cancer entities that varies from 35% to 60% [2, 4, 8, 12]. The so-called overexpression of p53, which comprises detectable p53 stabilized either through mutation or complex binding with certain proteins like heat-shock protein 70 or viral oncoproteins [18, 25], has been reported for squamous cell carcinoma of the head and neck with strong variations between 34% and 92% depending on materials and methods [2, 10, 13, 23, 32]. In a recent publication [29] a correlation between p53 overexpression and a worse survival rate due to an early incidence of second primaries and recurrences in patients with head and neck cancer was reported. These findings may have important implications for therapy decisions and especially for the follow-up of these patients.

A fairly new serological parameter are antibodies against the p53 protein. These antibodies were first described by Crawford and co-workers in patients with breast cancer in 1982 [6] and have been investigated since that time in many different tumor entities [1, 27]. The p53 antibodies seem to be an indicator for malignancy, since they are just found in cancer patients and only rarely in patients with non-malignant diseases [1, 22, 24]. The underlying mechanism that triggers antibody production against the p53 protein is still unknown, but p53 overexpression seems to be a prerequisite [16, 21, 27]. For patients with colon and breast cancer it has already been shown that the occurrence of p53 serum antibodies is an indicator for a worse prognosis [14, 26]. In this study we tried to determine if the occurrence of p53 antibodies in the serum of patients with head and neck cancer is of value as a

J.A. Werner (✉) · S. Gottschlich · B.J. Folz · T. Goeroegh
B.M. Lippert · J.-D. Maass · H. Rudert
Department of Otorhinolaryngology, Head and Neck Surgery, Christian-Albrechts-University, Arnold-Heller-Str. 14, 24105 Kiel, Germany
Fax: 0049 431 597–2272

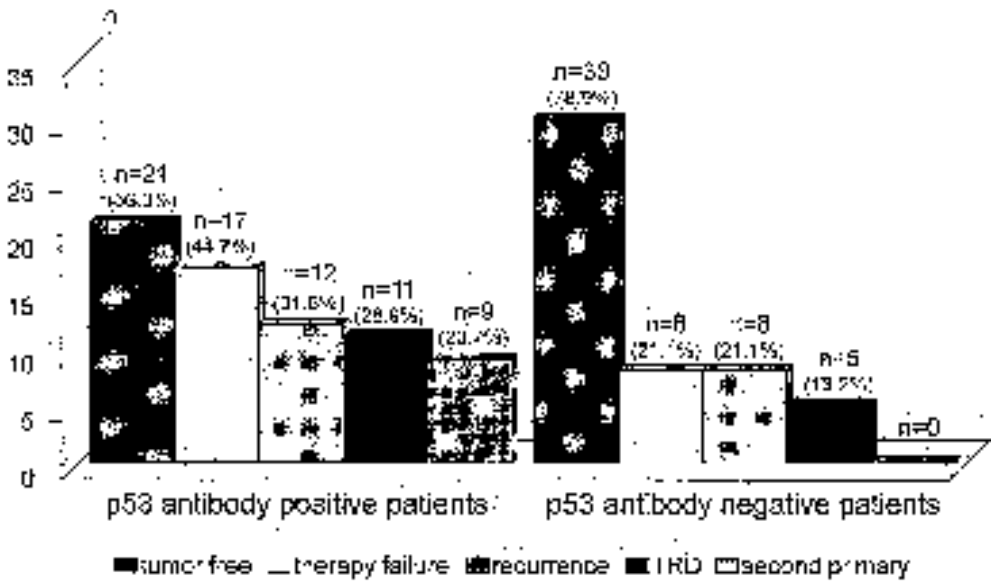

Fig. 1 Post-therapy course of 38 p53-antibody-seropositive and 38 p53-antibody-seronegative patients with head and neck cancer. Therapy failure is defined as recurrence and/or second primary tumor and/or tumor related death (TRD)

biomarker to identify patients who are at high risk of developing recurrences or second primary tumors and would benefit from a more intensive follow-up and preventive measures.

Materials and methods

The sera of 143 patients with a histologically proven squamous cell carcinoma of the head and neck were investigated for the presence of p53 antibodies. The tumors were located in the larynx (n = 50), hypopharynx (n = 34), oropharynx (n = 38), oral cavity (n = 11) and skin (n = 5); five tumors were lymph node metastases of squamous cell carcinoma of the head and neck with unknown primary. According to the 1987 TNM classification of the UICC, 29 patients were stage I, 18 stage II, 83 stage III and 13 stage IV. The mean age was 61.8 ± 11.3 years (range: 42–96 years). The sera of 41 healthy blood donors and 17 patients with chronic inflammatory diseases of the head and neck served as controls.

The blood was centrifuged at 2000 U/min directly after drawing and the serum was kept frozen at –80 °C until further investigation. An enzyme-linked immunosorbent assay (DIA 0301 E, Dianova, Hamburg, Germany), with recombinant wild-type p53 as antigen, was used for the p53 antibody determination. The photometric determination of the absorption was carried out with an automated microtiter plate reader (Dynatech, Chantilly, USA).

In addition the post-therapeutic course of the patients was observed in two matched subsets (n = 38 each) of patients that consisted of p53-antibody-seronegative (sn) and seropositive (sp) individuals. Each subgroup consisted of patients with similar or exactly the same characteristics or tumor characteristics. The median age of the patients in the seronegative group was 60.7 ± 10.6 years (range: 46–96 years) and in the seropositive group 60.9 ± 10.5 years (range: 46–90 years). Four women and 34 men were in each group. The tumors were located in the oral cavity (n = 7sp/7sn), the oropharynx (n = 7sp/7sn), the hypopharynx (n = 11sp/11sn), the larynx (n = 10sp/11sn), and the skin (n = 1sp/1sn) or were lymph node metastasis of cancers of unknown primary (n = 2sp/1sn). The primary therapy consisted of surgery and subsequent radiation therapy in all cases. The mean follow-up in the sp group was 24 months (range: 4–156 months) and, in the sn group, 20 months (range: 8–32 months). The course of these two patient groups was evaluated and compared in respect to recurrences, second primary tumors and tumor-related deaths beginning on the day of diagnosis. Patients with a tumor recurrence and /or second primary tumors and /or tumor-related death were summarized in one group as cases of therapy failure.

Results

p53 serum antibodies were detected in 39 of 143 patients (27.3%) with squamous cell carcinoma of the head and neck. The control sera of healthy blood donors (n = 41) and of patients with chronic inflammatory diseases of the head and neck (n = 17) did not show antibody reactivity against p53. The occurrence of p53 serum antibodies was not limited to certain tumor stages. Antibodies were found in advanced tumor stages as well as in stage I tumors. p53 antibodies were detected in 10/29 patients in stage I, 3/18 in stage II, 22/83 in stage III, and 4/13 in stage IV. No correlation of the tumor stage with the incidence of p53 serum antibodies was found. There was also no correlation of p53 antibody reactivity with tumor localisation, age or gender of the patient.

Two matched groups with 38 patients each, p53-antibody-seropositive and seronegative, were compared during their post-therapy follow-up. The outcome and course of the disease of the two groups were significantly different ($p < 0.05$). During the follow-up 12/38 seropositive and 8/38 seronegative patients developed local recurrences. Seven of the 12 p53-antibody-seropositive patients with a local recurrence and 1 of the 8 seronegative patients with a local recurrence died from tumor-related causes. In total, 11 tumor-related deaths were observed in the p53-antibody positive group, whereas 5 patients died from tumor-related causes in the seronegative group. Second primary tumors (n = 9) were exclusively observed in the seropositive group of patients. The second primaries were located in the oropharynx (n = 1), hypopharynx (n = 3), lung (n = 3), esophagus (n = 1), and the colon (n = 1). Of these 9 patients with second primary tumors, 2 developed a third primary tumor during the follow-up. One of these patients with a second

primary cancer in the esophagus developed colon cancer, and the other patient with a second primary located in the lung was diagnosed with a third primary in the kidney. When patients with recurrences, tumor-related deaths, and second primary tumors are summarized as a therapy-failure group and the incidences in seronegative and seropositive patients are compared, a significant difference ($p < 0.05$) was observed. In the seropositive group, 17 patients (44.7%) experienced therapy failure, whereas, in the seronegative group, only 8 cases (21.1%) of therapy failure occurred (Fig. 1).

Discussion

Mutations of the p53 tumor-suppressor gene, as well as the overexpression of p53 protein in tumor tissue, and the occurrence of serum p53 antibodies have been demonstrated in a variety of human malignancies. Since the first report on p53 by Linzer and Levine [19] the important role of the p53 protein for the normal function of the cell has been elucidated. The p53 protein has been implicated in DNA repair and synthesis, cell differentiation and apoptosis. Among its many functions is the regulation of the cell cycle through transcription modulation of genes like *GADD45* [15] or *mdm-2* [34]. The function of the p53 protein as a "guardian of the genome" is generally accepted [17].

Why and how a nuclear protein like p53 induces a B cell response is still unclear. Two major mechanisms have been proposed to explain how p53 reaches immunogenicity. The first involves the p53 protein becoming immunogenic through mutation of the gene with subsequent changes in specific protein determinants. This assumption is based on observations by Winter and co-workers [33], who reported p53 antibody seropositivity only in lung cancer patients with a mutation in exon 7, whereas patients with mutations in exon 8 did not show antibodies. A slight variation of this hypothesis was postulated for the development of p53 antibodies in breast cancer patients by Davidoff and co-workers [7]. The authors reported that the immune response against p53 protein is dependent on complex binding between heat-shock protein 70 and p53, triggered by exon 5 and 6 mutations of the p53 gene. The second main hypothesis assumes that a pure accumulation of p53 protein causes the antibody production [21]. Cell necrosis, releasing p53 from its natural compartment and thus presenting it to the humoral response system, may be another possible mechanism in the immunisation process. Schlichtholz and co-workers [17] and Labrecque and co-workers [16] showed that p53 antibodies recognize specific epitopes of the p53 protein at the amino-terminal end and at the carboxy-terminal end. Since more than 90 % of p53 mutations are located in the highly conserved regions in the middle of the gene (amino acids 81–318), it seems that these mutations do not influence the immunogenicity of the p53 protein. A recent investigation [11] of head and neck cancer patients demonstrated no correlation between a p53 immune response and certain exon mutations of the p53 gene, thus supporting the accumulation theory postulated by Schlichtholz and co-workers and Labrecque and co-workers. Peyrat and co-workers [26] reported a worse survival rate for p53-antibody-positive breast cancer patients undergoing locoregional surgery. A similar prognostic relevance of p53 serum antibodies was postulated by Houbiers and co-workers for patients with colon cancer [14].

The results of this study indicate that p53 serum antibodies seem to be of prognostic value for patients with head and neck cancer also. The number of second primary tumors ($n = 9$) was significantly higher in the seropositive group than in the seronegative group ($n = 0$). The fairly short average follow-up of 2 years already reveals a significant difference between the two groups regarding tumor-related deaths and the number of recurrences. These results show that the occurrence of serum p53 antibodies may be a strong predicting factor for therapy failure in patients with head and neck cancer.

A recent investigation [29] showed that the overexpression of p53 in tumor tissue of patients with head and neck cancer may be an indicator for an increased number of recurrences and second primaries in these patients. Although the absolute number of second primaries was not significantly higher, the time of second primary development was significantly shorter in the p53-positive group and the overall survival reported in this study was worse for p53-positive patients. Other investigations did not find p53 overexpression of prognostic value in head and neck cancer [9]. Lowe and co-workers [20] assume that p53 overexpression negatively influences the response to chemotherapy in cancer, whereas Bradford and co-workers [3] describe a good response to chemotherapy and radiation therapy in patients with laryngeal carcinomas overexpressing p53.

Although the underlying mechanism of the immune response against p53 protein is still unclear, the p53 overexpression in tumor tissue seems to be a prerequisite for the production of p53 antibodies [16, 21, 27]. Only rarely are p53 antibodies found in individuals without known malignant tumor [1, 24]. Therefore, the prognostic value of p53 overexpression in tumor tissue of patients with head and neck cancer, postulated by Shin and coworkers [29], can support the findings of the presented study. Similar results during the 2-year follow-up were obtained for serum p53 antibodies in head and neck cancer patients. The annual rate of second primary tumors within 2 years in the seropositive group was significantly higher than the expected average rate of 4%–7% per year [31]. The patients who had serum p53 antibodies showed a higher percentage of recurrences and tumor-related deaths than did the seronegative cancer patients. The high incidence of treatment failure reported for patients with p53 overexpression [29] may thus also be true for patients with serum p53 antibodies. It is possible that the detection of serum p53 antibodies could identify patients with an even higher risk of developing second primaries, as is assumed when p53 overexpression is detected in tumor tissue alone. Prognostic relevance of

p53 overexpression has also been found in patients with breast cancer [30], but without any corresponding occurrence or predictive value of p53 antibodies.

The results of this study indicate that the occurrence of serum p53 antibodies in patients with head and neck cancer may serve as a biological marker to identify patients that are at high risk of developing recurrences and/or second primary tumors. Compared to the determination of p53 overexpression in tumor tissue, the determination of serum p53 antibody levels is faster, less laborious, and less expensive. It may even be more reliable, since a recent study demonstrated the existence of strong variations of intratumoral p53 staining in head and neck tumors [28]. It is also known that p53 overexpression detected by immunohistochemistry does not always represent mutant p53, but can also imply stabilisation of wild-type p53 [18, 25]. The detection of serum p53 antibodies may help to select patients that would benefit from an intensive and close follow-up or from chemoprevention. This way a more specific follow-up regime for head and neck cancer patients could be developed.

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