Antibodies to P53 may Predict a High Risk of Treatment Failure in Head and Neck Cancer

J.D. Maass, Junior House Officer, **S. Gottschlich**, Senior House Officer,
B. M. Lippert, Senior Register, **C. Kuelkens**, Senior House Officer,
B. J. Folz, Senior House Officer, **J. A. Werner**, Vice Chairman,
 Dept. of Otolaryngology & Head and Neck Surgery, University of Kiel,
 Arnold-Heller-Str. 14, 24105 Kiel, Germany

Overexpression of p53 as well as the occurrence of serum p53 antibodies have been correlated to shorter survival and relapse-free intervals in head and neck squamous cell carcinoma (HNSCC). The aim of this study was to reconsider the clinical implications of serum p53 antibodies in HNSCC. Two matched study groups (n=78 patients with HNSCC each), one group positive and one negative for serum p53 antibodies, were followed-up (median of 3 years) regarding the occurrence of relapses, second primaries and tumor related deaths. Tumor relapses (n= 27 vs. 12), second primaries (n= 16 vs. 4) and tumor related deaths (n=21 vs. 9) occurred more frequently in the seropositive group. The results of this study indicate that the presence of serum p53 antibodies in patients with HNSCC points out a group of patients, who have a high risk of treatment failure.

Introduction

p53 overexpression is commonly observed in squamous cell carcinoma of the head and neck⁹ (HNSCC), representing either complex binding and stabilization of wild-type p53¹⁰ or mutant forms of p53. Among its many functions, p53 takes part in the regulation of cell cycle arrest⁷, thus enabling cell mechanisms to repair damaged DNA. The clinical potential of p53 overexpression has been discussed controversially for HNSCC. While some authors do not find any correlations with clinical parameters like stage of the disease or tumor differentiation¹⁶, others do consider p53 overexpress as a prognostic indicator for early recurrences and the increased development of second primary tumors¹⁴.

In 1982, antibodies against p53 were first

described by Crawford and coworkers in sera of breast cancer patients. Since then, its diagnostic and prognostic applicability has been investigated for a variety of different epithelial tumors¹, including HNSCC^{2, 8, 17}.

It was postulated that the detection of serum p53 antibodies in HNSCC may be associated with a high probability of treatment failure^{2, 6}. In a more recent article the prevalence of serum p53 antibodies was linked to an elevated probability for the early development of second primary tumors in HNSCC patients¹⁷. Therefore, the aim of this study was to investigate the prognostic potential of serum p53 antibodies in HNSCC in order to certify or deny the clinical implications that have been described so far.

MATERIAL AND METHODS

Two matched study groups with histologically proven HNSCC (78 patients each) were clinically followed-up for 3 years after initial therapy. The first study group comprised patients without serum p53 antibodies, the second group comprised HNSCC patients with serum p53 antibodies. The two groups were pair-matched regarding tumor location, differentiation and stage of the tumor, as well as age and gender (68 male, 10 female) of the patients. The age of the patients was 61.3 ± 10.5 years in the seronegative group and 60.8 ± 10.1 years in the seropositive group. The tumors were located in the oral cavity (n=13), oropharynx (n=14), hypopharynx (n=23), and larynx (n=28). According to the UICC (1978), the stage of the tumors was classified as follows. Ten tumors were stage I, 13 tumors were stage II, 37 were stage III and 18 were stage IV. All patients

were treated with surgery followed by radiotherapy. The patients were followed-up every one to three months.

RESULTS

Two groups of patients with and without serum p53 antibodies (78 patients each) were followed-up clinically for 3 years (5-134 months). The clinical development after initial therapy differed in the two groups. Within the follow-up, 27 seropositive and 12 seronegative patients developed tumor recurrences. Tumor related deaths occurred 21 times in the seropositive and

postulated and promoted as prognostic factors, amongst them the overexpression of p53¹⁴ and the detection of serum p53 antibodies^{2, 6, 17}.

The overexpression of p53 was investigated in numerous studies in a variety of cancer types. Its prognostic potential in HNSCC with special regard to therapy success [14, 2], and its clinical applicability as an aid in the planning of treatment strategies has been discussed controversially. Yin and coworkers¹⁸ described a shorter overall survival and shorter relapse-free intervals for patients with p53-positive HNSCC. Shin and coworkers¹⁴ have shown, that the overexpression of p53 in HNSCC is associated with an early occurrence of second primary tumors and tumor relapse, both limiting factors of survival. In contrast, Bourhis and coworkers² did not find such an association in HNSCC.

p53 antibodies were first described by Crawford et al. in patients with breast cancer³ and have since been detected in a variety of different kinds of tumors¹. They seem to be an indicator for malignancy, since they have been found in cancer patients exclusively, with rare exception^{1, 8}. Peyrat et al.¹¹ reported a worse postsurgical survival rate for breast cancer patients, who were seropositive for p53 antibodies. A similar prognostic relevance of serum p53 antibodies was postulated for patients with colon cancer⁵. In contrast, it has been shown recently that in lung cancer the presence of serum p53 antibodies was not associated with any clinical parameter or prognostic markers¹².

It has not been clarified yet how p53 becomes immunogenic and why some tumors that overexpress p53 lead to the development of p53 antibodies while others don't⁸. p53 may be released in the course of tumor necrosis, thus leading to a B-cell response; on the other hand it has been proposed that p53 accumulation within the cell may lead to a self immunisation process against p53¹³.

Recent studies demonstrated an increased risk of relapse and death for patients with head and

Table I

Patients with and without serum p53 antibodies (78 patients each) were followed-up and compared regarding the occurrence of relapses, second primary tumors and tumor related deaths. The mean follow-up time was 3 years. Relapses, tumor related deaths and second primaries occurred more often in the seropositive group.

	p53-Antibody positive	p53-Antibody negative
Relapses	34.6% (n=27)	15.4% (n=12)
Tumor Related Deaths	26.9% (n=21)	11.5% (n=9)
Second Primaries	20.5% (n=16)	5.1% (n=4)

9 times in the seronegative group. Second primary tumors were diagnosed 16 times in the seropositive and 4 times in the seronegative group (Table I). No correlations were seen between the detection of serum p53 antibodies and tumor qualities such as size of the tumor, nodal involvement and metastatic invasion. The histological classification of the tumors did neither bear any correlation with p53 immunogenicity.

DISCUSSION

Limiting factors for long-term survival of head and neck cancer patients are tumor expansion into vital structures, the occurrence of relapses and second primary tumors, and the manifestation of distant metastases. Over the last few years many biological markers have been

neck cancer who were seropositive for p53 antibodies^{2,17}. These findings could be valuable for therapy decisions and for the follow-up of these patients; p53 antibodies may even represent a parameter for the identification of patients with a poor prognosis⁶. The detection of p53 antibodies may identify patients that are at high risk to develop early recurrences or second primary tumors and who would subsequently benefit from a more intensive clinical follow-up and preventive measures. It was the aim to verify or deny these findings with a large study group.

In this study, the results of former studies^{2,17} could be confirmed. The annual rate of second primaries was significantly higher in the seropositive group than the expected rates of approximately 5% per year⁴. The seropositive patients showed a higher degree of recurrences and tumor related deaths than seronegative

cancer patients. These results indicate and verify that the detection of serum p53 antibodies in HNSCC may identify a group of patients, who have a high risk of therapy failure.

Compared to the determination of p53-overexpression in tumor tissue, the determination of serum p53-antibody levels is time saving, less invasive for the patient and less expensive. Moreover, the detection of serum p53 antibodies in HNSCC seems to be more reliable for a prognostic outlook than the determination of p53 protein in tumor tissues. The detection of serum p53 antibodies may help to select patients who benefit from an intensive and close follow-up or from chemoprevention. The detection of serum p53 antibodies may enable us to develop more specific therapies and follow-up regimes for certain head and neck cancer patients.

References

1. Angelopoulou, K., Diamandis, E.P., Sutherland, D.J.A. et al. (1994) : Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. *International Journal of Cancer* 58 : 480-487.
2. Bourhis, J., Lubin, R., Roche, B. et al. (1996) : Analysis of p53 serum antibodies in patients with head and neck squamous cell carcinoma. *Journal of the National Cancer Institute* 17 : 1228-1223.
3. Crawford, L.V., Pim, D.C., Bulbrook, R.D. et al. (1982) : Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *International Journal of Cancer* 30 : 403-408.
4. Hong, W.K., Bromer, R.H., Amato, D.Å. et al. (1985) : Patterns of relapse in locally advanced head and neck cancer patients who achieved complete remission after combined modality therapy. *Cancer* 56 : 1242-1245.
5. Houbiers, J.G.A., van der Burg, S.H., van de Watering, L.M.G. et al. (1995) : Antibodies against p53 are associated with poor prognosis of colorectal cancer. *British Journal of Cancer* 72 : 637-641.
6. Lavielle, J.P., Lubin, R., Soussi, T. et al. (1996) : Analysis of p53 antibody response in patients with squamous cell carcinoma of the head and neck. *Anticancer Research* 16 : 2385-2388.
7. Levine, A.I., Perry, M.E., Chang, A. et al. (1994) : The Walter Huber Lecture : The role of the p53 tumour-suppressor gene in tumorigenesis. *British Journal of Cancer* 69 : 409-416.
8. Maass, J.D., Gottschlich, S., Lippert, B. M. et al. (1996) : Antikörperbildung gegen das zelluläre Protein p53 bei Patienten mit Plattenepithelkarzinomen der oberen Luft- und Speisewege. *Laryngo-Rhino-Otologie* 75 : 53-56.
9. Maass, J.D., Gottschlich, S., Goeroegh, T. et al. (1997) : High rate of p53 overexpression in head and neck carcinomas detected with a refined ELISA. *Anticancer Research* 17 : 473-478.
10. Momand, J., Zambetti, G.P., Olson, D.C. et al. (1992) : The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 69 : 1237-1245.
11. Peyrat, J.P., Bonnetterre, J., Lubin, R. et al. (1995) : Prognostic significance of circulating p53 antibodies in patients undergoing surgery for locoregional breast cancer. *Lancet* 345 : 621-622.
12. Rosenfeld, M.R., Malats, N., Schramm, L., et al. (1977) : Serum anti p53 antibodies and prognosis of patients with small cell lung cancer. *Journal of the National Cancer Institute* 89 : 381-384.
13. Schlichtholz, B., Legros, Y., Gillet, Y. et al. (1992) : The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Research* 52 : 6380-6384.

14. Shin, D.M., Lee, J.S., Lippmann, S.M. et al. (1996) : p53 expression : Predicting recurrence and secondary primary tumors in head and neck squamous cell carcinoma, *Journal of the National Cancer Institute* 88 : 519-529.
15. UICC, Union Internationale Contre le Cancer, TNM-Klassifikation maligner Tumoren. 4, Aufl., Springer, Berlin (1987).
16. Warnakulasuriya, K.A., Johnson, N.W. (1992) : Expression of p53 mutant nuclear phosphoprotein in oral carcinoma and potentially malignant oral lesions. *Journals of Oral Pathology and Medicine* 21 : 404-408.
17. Werner, J.A., Gottschlich, S., Folz, B.J. et al. (1977) : p53 serum antibodies as prognostic indicator in head and neck cancer. *Cancer Immunology Immunotherapy* 44 : 112-116.
18. Yin. X.Y., Smith, M.L., Whiteside, T.L. et al. (1993) : Abnormalities in the p53 gene in tumors and cell lines of human squamous cell carcinomas of the head and neck. *International Journal of cancer* 54 : 322-327.

Announcement

BMJ-MAHE Medical Writing Workshop

March 2-3, 1998, Manipal

The Manipal Academy of Higher Education, a deemed university, which is associated with the Indian edition of the British Medical Journal, is organizing a national workshop on medical writing. Faculty for the workshop will include the BMJ Editor, Dr Richard Smith, other members of the BMJ editorial staff and leading Indian editors and medical writers. Topics will include :

- What editors want
- Scientific structure of an article
- How to write a good article
- Refereeing and editing
- Medical writing in India
- Ethical issues in medical writing
- Use of the electronic media

The workshop will include small group tutoring sessions in which delegates may discuss their own scientific papers with the workshop faculty.

For further details please contact Dr. GG Christo, Executive Editor, BMJ Indian Edition, University Building, Manipal Academy of Higher Education, Manipal 576119. Ph 08252 71201, Fax 08252, 70061.