European Journal of Cancer, Vol. 34, No. 6, pp. 941–942, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

Letters

PII: S0959-8049(97)10100-9

Limitations of *p53* Gene Intron 6 *MSP1* Restriction Fragment Length Polymorphism Analysis

J. Pavelić, M. Herak Bosnar and K. Gall-Trošelj

Division of Molecular Medicine, Laboratory of Molecular Oncology, Ruder Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia

VARIATION IN the nucleotide sequence of the human genome is common, occurring approximately once every 200 base pairs. This renders the human population dimorphic for certain DNA sequences. If a difference in the DNA sequence occurs within the recognition sequence of a restriction enzyme, the fragments produced by that restriction enzyme will be of different lengths in different people. This can be recognised by the so-called restriction fragment length polymorphism (RFLP) method.

The loss of tumour suppressor genes appears to be involved in the pathogenesis of many solid tumours. It can be detected, among other methods, by a loss of heterozygosity (LOH) based on the use of the RFLP approach. When small amounts of starting material are available, polymerase chain reaction (PCR) in combination with restriction endonuclease digest is recommended for RFLP/LOH determination. However, due to the fact that polymorphism is limited as only two possible alleles exist, the heterozygosity (informativity) rate of this method is limited as well.

Alterations in the *p53* gene are among the most common changes observed in human malignancies. One of the alleles is frequently lost, whilst the other is subject to additional mutations. When this genetic loss occurs in a tumour, it is usually from one rather than from both homologues and the loss of heterozygosity can be detected.

Several different *p53* markers are currently in use for *p53* gene allelic loss screening: a codon 72 (exon 4) *Bst*UI restriction site, an *MSP1* restriction site in intron 6, VNTR marker in intron 1, 16 bp duplication in intron 3, and several microsatellite markers in different gene regions [1–7]. Based on our own literature data, codon 72 *Bst*UI RFLP seems to be acceptable for the LOH assay. According to literature data, heterozygosity at this locus varies from 30.2 to 63.6% [3, 5, 8, 9]. Based on 125 unrelated Croatians tested in our laboratory, the observed heterozygosity was 36.8%.

Correspondence to J. Pavelić.
Received 7 Aug. 1997; accepted 15 Oct. 1997.

In 1991, McDaniel and colleagues [4] described another restriction site (intron 6/*MSP1*), which they suggested to be suitable for LOH testing. The heterozygosity observed in 57 unrelated Americans was 46%. The frequency of alleles was 74 and 26% for alleles with and without a specific endonuclease restriction site. Additional literature data about heterozygosity at this locus and its suitability for LOH investigations are limited, thus providing us with no exact data about this locus informativity [2] or showing between 11 and 21% of heterozygous cases [1].

In our laboratory, research is focused on the detection of *p53* gene alterations and their involvement in tumour development and progression. Apart from codon 72, the intron 6 *MSP1* restriction site has been used for loss of heterozygosity analyses. Unfortunately, the intron 6 locus has not revealed the expected heterozygosity, described originally by McDaniel and colleagues [4]. So far, we have tested 95 samples of normal tissues (whole blood) obtained from the same number of unrelated Croatians (Figure 1). Informativity was only 17.9%, a large departure from what was originally suggested. Even the frequency of alleles (among 78 homozygous samples) was different from that which was previously described. The frequency of the allele containing the restriction site was 96%; the other allele appeared only sporadically, with a frequency of 4%. The observed differences could be, at least partially, ascribed to ethnic differences (Americans, no race indicated, versus central Europeans) as has been described by Sjalander and associates [1], Wu and colleagues [3] and Kageyama and associates [8]. However, with regard to our own data, the data of other authors who have studied this restriction locus [2] and the fact that the data in literature on the use of this marker are poor, we believe that it is simply a matter of a lack of informativity at the intron 6 *MSP1* locus which renders the determination of the deletion of the gene (chromosome) *p53* unsuitable.

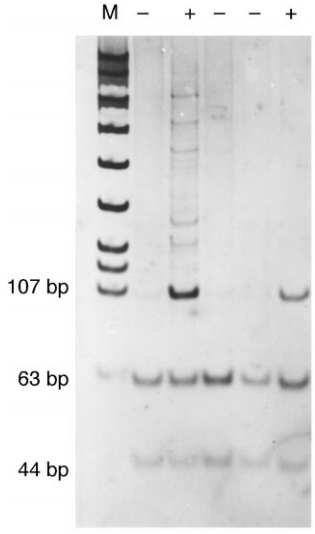

Figure 1. Informativity status (restriction fragment length polymorphism analysis) at intron 6 *MSP1* region of gene *p53* in human normal tissue. M, DNA marker VIII (Boehringer Mannheim); +, informative (heterozygous; one allele with no restriction site—107 base pairs; and another with restriction site present—bands at 63 and 44 base pairs; –, not informative (homozygous; both alleles with restriction site) (non-denaturing polyacrylamide gel, 30 × 30 cm, 1 mm thick, 6 h electrophoresis, 50 W).

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Acknowledgement—This work was supported by grants (00981103, 00981104 and 101751) from the Ministry of Science and Technology, Republic of Croatia.

European Journal of Cancer, Vol. 34, No. 6, pp. 942–943, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(97)10110-1

Autoantibodies to the 90 kDa Heat Shock Protein and Poor Survival in Breast Cancer Patients

S.E. Conroy,[1] P.D. Sasieni,[2] I. Fentiman[3] and D.S. Latchman[1]

[1]Medical Molecular Biology Unit, Department of Molecular Pathology, Windeyer Institute of Medical Sciences, University College London Medical School, The Windeyer Building, 46 Cleveland Street, London W1P 6DB; [2]Imperial Cancer Research Fund, Department of Mathematics, Statistics and Epidemiology; and [3]Imperial Cancer Research Fund, Clinical Oncology Unit, U.K.

Correspondence to D.S. Latchman.
Received 26 Aug. 1997; accepted 30 Oct. 1997.

FOLLOWING EARLIER reports of poor survival in breast cancer patients overexpressing the human 90 kDa heat shock protein (hsp90) [1], we previously tested sera, taken after cancer diagnosis, for the presence of autoantibodies to this protein [2]. In that study, we found that autoantibodies to hsp90 were detectable at high frequencies in breast cancer patients, but not in normal controls. Moreover, the presence of autoantibodies ($P < 0.04$) and the presence of involved nodes ($P < 0.001$) correlated with the development of metastases, with the presence of antibodies to hsp90 and positive nodes being more clearly correlated with subsequent metastatic recurrence than nodal involvement alone [2]. However, there were insufficient clinical follow-up data to investigate whether the presence of antibodies to hsp90 at the time of diagnosis related to overall survival in these patients.

Here we report on the survival of 327 women with breast cancer diagnosed between January 1980 and April 1984 who were treated at Guy's Hospital, London, U.K. Sera had been taken both pre- (1–2 days before diagnostic excision biopsy) and post- (8–10 days after) surgery. The women are under active follow-up. All but 3 women were followed for a minimum of 10 years or until death. To date there have been 130 deaths attributed to breast cancer and 17 other deaths. The latter were treated as censoring events in all analyses. Sera, which had been stored at −20°C were tested for the presence of antibodies to hsp90 using an enzyme linked immunosorbent assay (ELISA) developed in our laboratory [3]. The positivity rates for the 214 presurgery and 200 postsurgery samples available were 63 and 64%, respectively.

Of the 87 women with both pre- and postsurgery antibody tests, 66 had the same result on both samples (kappa +0.48, $P < 0.0001$). The average breast cancer mortality rate was greater in women testing positive for antibodies to hsp90 than those testing negative, but the difference in mortality between the two groups based on presurgery sera was minimal (42 per 1000 women years versus 43, $P = 0.95$ log rank). The difference in mortality rates per 1000 women years based on postsurgery results was greater with a clear increase in mortality rate in women with antibodies to hsp90 (34 in women with no antibodies to hsp90 versus 45 women with antibodies to hsp90), but was still not significant ($P = 0.25$) (Table 1). Women with antibodies to hsp90 showed an increased number of deaths compared with that expected if survival was independent of the presence of antibodies, whilst women without antibodies showed a decreased number of actual deaths compared with expected (Table 1). Using a Cox model, the hazard ratio (95% confidence interval) associated with hsp90 antibodies in sera postsurgery was 1.32 (0.8–2.1; $P = 0.56$); after adjustment for age, menstrual status, nodal status, tumour size, grade and histology it was 1.37 (0.8–2.3; $P = 0.30$). Interestingly, the hazard ratio associated with hsp90 antibodies was 1.00 for the first 5 years and 1.84 thereafter. This suggests that the development of antibodies to hsp90 around the time of surgery has a particular influence on long-term survival.

Thus, there appears to be an association between mortality rates and antibodies to hsp90. However, this study was conducted on women with relatively good prognosis—80% were still alive 5 years after diagnosis, 66% after 10 years. Future studies will need to be carried out on cohorts with more breast cancer deaths in order to determine whether the lower mortality rate in women without hsp90 antibodies in their sera is reproducible and of clinical relevance.

Table 1. *Survival of patients with or without antibodies to hsp*90 *in samples taken pre- or postsurgery*

	Presurgery				Postsurgery			
hsp90 antibodies	Frequency	Average death rate per 1000 women years	Observed deaths	Expected deaths	Frequency	Average death rate per 1000 women years	Observed deaths	Expected deaths
Negative	79	42	32	32.3	73	34	25	30.0
Positive	135	43	57	56.7	127	45	54	49.0
ND	113	37		$P = 0.95$*	127	40		$P = 0.25$*

ND, not determined. **P* value based on log rank test (excluding women whose antibody status was not determined).

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Acknowledgement—We thank the BUPA Medical Foundation and the Imperial Cancer Research Fund for supporting this work.

European Journal of Cancer, Vol. 34, No. 6, pp. 943–944, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(97)10099-5

Is There a Place for Embolisation in the Treatment of Non-Hodgkin's Lymphoma?

E. Gamelin,[1] I. Pellier,[2] A. Pasco,[3] X. Rialland[2] and N. Ifrah[4]

[1]Centre Paul Papin; [2]Unité d'Hématologie-Oncologie Pédiatrique; [3]Service de Radiologie; and [4]Service des Maladies du Sang, Centre Hospitalier Universitaire, Angers, 4 rue Lancy, 49033 Angers Cédex, France

LOCAL RECURRENCE of high-grade non-Hodgkin's lymphoma (NHL) is usually treated with intensive chemotherapy and/or radiotherapy [1, 2]. When these modalities are inadequate or no longer possible, selective arterial embolisation can be an alternative treatment to radiotherapy and chemotherapy. We report here a case history.

A 64-year-old woman was hospitalised in November 1992 for generalised polyadenopathy (supraclavicular, axillary and mediastinal) with hepatosplenomegaly, fever, sweat without weight loss. Her WHO performance status was grade 2. Blood counts were normal. The following laboratory values were noted: lactate dehydrogenase 670 U/l, fibrinogen 6 g/l, hypogammaglobulinaemia 6 g/l. Bone marrow was not involved. Diagnosis, obtained from biopsy of supraclavicular lymph nodes, was a large B lymphoma according to REAL classification [3], stage III B LNH (Ann Arbor classification).

Initial therapy consisted of alternating cycles with either vindesine, epirubicin, cyclophosphamide, and prednisone, or ifosfamide, etoposide, methotrexate, and mitoxantrone chemotherapy. Although initial symptoms disappeared rapidly, relapse occurred in June 1993, on therapy, involving clavicle and adjacent soft tissue. Local irradiation (40 Gy) was followed by alternating cycles of ifosfamide, methotrexate, etoposide and lomustine, etoposide, cyclophosphamide, plus prednisone chemotherapy, administered until November 1993. At this time, the patient had a complete response.

Four months later, a painful mass reappeared in the same supraclavicular right region and did not respond to three cycles of vinorelbine. Its size was approximately 5 cm diameter, involving the supraclavicular lymph node region, with clavicular tumefaction. Subclavian right arteriography with a Head-Hunter catheter showed that the mass was hypervascular. In contrast, vascularisation of the bone clavicular tumour was not increased. A superselective thoraco-acromial arterial catheterisation was carried out with a microcatheter, and embolisation of this vessel was successfully achieved using Ivalon particles. Vascularisation of the cervical mass decreased by 67%, without any incidence of catheter blockage. The pain disappeared and the size of the tumour dramatically decreased within a few days. Three weeks later, recurrent splenomegaly was noted. Chemotherapy with etoposide, cisplatin, cytarabine, and methylprednisolone, followed by three cycles of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), then a cyclic alternating consolidation schedule with three courses of etoposide, cyclophosphamide, prednisone, vincristine, doxorubicin, and three courses of vincristine, dexamethasone, doxorubicin achieved complete remission. In June 1997, the patient was alive, with no evidence of lymphoma.

We think that embolisation may have played a part in achieving and maintaining complete response of the lymphoma. After radiation therapy, chemotherapy was inadequate and embolisation was the only local treatment which could be applied for this patient. To our knowledge, this technique has not been reported in the literature. However, we suggest that in such cases, it could be an alternative for adjuvant therapy of hypervascularised tumours, especially if radiotherapy is no longer possible.

Correspondence to N. Ifrah.
Received 9 Oct. 1997; accepted 28 Oct. 1997.

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