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PTEN expression is a strong predictor of survival in mesothelioma patients☆

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Abstract

Background: Malignant pleural mesothelioma (MPM) is a highly aggressive tumour with poor prognosis and limited response to therapy. MPM is characterised by complex chromosomal aberrations, including chromosome 10 losses. The tumour suppressor gene phosphatase and tensin homologue deleted from chromosome 10 (PTEN) located on chromosome 10q23 plays an important role in different cancer, but its relevance for MPM is unclear. **Patients and methods:** In the present tissue microarray-based study, 341 MPM were studied for PTEN expression by immunohistochemistry using a monoclonal mouse PTEN antibody. Expression levels were semiquantitatively scored (negative, weak, moderate, strong). Expression of PTEN was correlated to overall survival. **Results:** Clinical data from 206 patients were available. One hundred and five patients were stage T4 and 92 patients presented with regional and mediastinal lymph node metastasis. Loss of PTEN expression was observed in 62% of the cases. The survival time was correlated to PTEN expression in 126 cases with complete follow-up data. Comparing any PTEN expression versus no expression, median survival time was significantly longer (log rank test $p = 0.0001$) in patients with PTEN expression (15.5 months; 95% CI: 3.8; 27.2 vs 9.7 months; 95% CI: 7.9; 11.7). Cox regression analysis revealed an association between PTEN expression and survival ($p = 0.003$) independently from the histological subtype ($p = 0.7$). **Conclusion:** PTEN is an independent prognostic biomarker in mesothelioma patients. The frequent loss of expression of the tumour suppressor gene PTEN suggests involvement of the PI3K-AKT/protein kinase B (PKB) pathway in MPMs, which may be relevant for future mesothelioma treatment.

Keywords: Malignant pleural mesothelioma; Tissue microarray; PTEN; Immunohistochemistry; Survival

1. Introduction

Malignant pleural mesothelioma (MPM) is a fatal disease with an increasing incidence in the United States and Western Europe until approximately 2020 [1]. Without treatment, the majority of patients will die within 6—18 months [2]. Response to standard chemotherapy is moderate, the greatest treatment success is currently reached by multimodal treatment offering a median survival of 23 months [3]. Patients with an epithelioid subtype are known to experience a longer survival in comparison to patients with other subtypes (sarcomatoid, biphasic) [4]. But nevertheless, there is a strong variability in patient survival and therapy response that is not explained only by tumour stage and histological subtype and may be due to biological variability. As a consequence of asbestos exposure, the major risk factor for the development of MPM [5], a chronic inflammation process occurs that leads over a long latency period to tumour development where different growth factors, signalling molecules, oncogenes and tumour suppressor genes have been hypothesised to be involved [6]. Therapeutic approaches targeting these signal transduction cascades may control MPM tumour cell growth and proliferation.

The tumour suppressor gene phosphatase and tensin homologue deleted from chromosome 10 (PTEN), also known as MMAC1 (mutated in multiple advanced cancers) or TEP1 (TGFβ-regulated and epithelioid cell-enriched phosphatase), is located on chromosome 10q23 and encodes protein regulating various signal transduction pathways and modulating cell growth processes, cell migration and apoptosis [7]. Mutations of this gene have been identified in a large fraction of tumours, including gliomas, endometrial cancers, breast, thyroid, bladder, ovary, small cell lung cancer and haematological malignancies [8]. In MPM, chromosome 10

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losses have been detected by comparative genomic hybridisation [9], therefore analysis of PTEN expression located on 10q23 might elucidate an interesting factor of mesothelioma carcinogenesis.

Consequently, the aim of the underlying study was to screen a large tissue bank consisting of 341 formalin-fixed, paraffin-embedded MPM biopsy specimens for the expression of PTEN, and to analyse the prognostic relevance of PTEN expression.

2. Patients and methods

2.1. Patients

All malignant mesotheliomas, diagnosed between 1975 and 2004, were retrieved from the archives of the Zurich Pneumoconiosis Research Group, Switzerland (Director: M. Rueegger). The total of 341 cases comprised 112 epithelioid, 183 biphasic and 46 sarcomatoid types. The tissue specimens were mainly derived from post-mortem examination (77% autopsy, 23% biopsy) and had uniformly been formalin-fixed and paraffin-embedded. They had all been originally examined and classified for the histological subtype by one experienced lung pathologist (P.V.) and were reviewed (M.H.) to identify suitable areas for tissue microarray construction.

2.2. Tissue microarray construction

The construction of a set of three tissue microarrays was accomplished with a custom-made, semiautomatic tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA) as described previously [10].

2.3. Immunohistochemistry

PTEN immunohistochemistry was first established on a multi-tumour-TMA consisting of the most frequent human tissues and carcinomas. 4.5 μm sections of tissue microarray blocks were transferred to an adhesive-coated slide system (Instrumedics, Hackensack, NJ, USA) supporting the cohesion of 0.6 mm array elements on glass. De-paraffined sections were automatically stained with BenchMark (Ventana, USA) using the iView DAB Detection kit (Ventana, USA).

The primary antibody for the expression of PTEN was a mouse monoclonal antibody (Novocastra, Sweden) diluted 1:200 and incubated for 30 min. PTEN expressing endometrioid adenocarcinoma of the uterus was used as a positive control, because PTEN is frequently inactivated by mutation and deletion in endometrioid carcinomas.

2.4. Data assessment and statistical analysis

The sections were semiquantitatively assessed for the cytoplasmatic PTEN expression by one observer (A.S.). Intensity of staining was scored semiquantitatively 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). Clinical data of these patients were retrospectively assessed from medical archives of the different hospitals and the local cancer registries. Statistical analysis was performed using Kaplan–Meier curves for correlation of survival time with expression of PTEN. For correlation between ordinal variables (including dichotomised variables: epithelioid vs non-epithelioid correlated to PTEN expression score), we used the gamma-coefficient. The joint influence of both predictors on survival was assessed by Cox regression analysis.

Table 1
TNM staging according to IMIG [11]

	No. of patients (%)
T-stage	
1	1 (0.5%)
2	29 (14%)
3	13 (6%)
4	105 (51%)
Unknown	58 (28.5%)
N-stage	
0	10 (5%)
1	43 (21%)
2	31 (15%)
3	18 (9%)
Unknown	104 (50%)
M-stage	
0	10 (5%)
1	100 (49%)
Unknown	96 (46%)

3. Results

Clinical data were assessed for a total of 206 patients (94% male) with a median age of 63 years (39; 97). Exposure to asbestos was known in 97 cases (47.1%). Disease was located in 52% of the patients on the right side. The histological subtype was epithelioid in 63 (31%) patients, in 109 (52%) a biphasic and in 34 (17%) patients a sarcomatoid. As tumour stage was not documented in every case, a retrospective staging was performed based on pathology reports' description according to the IMIG staging system [11]. Complete tumour stage is summarised in Table 1. Treatment was in 61 cases (30%) surgical and comprised 27 extrapleural pneumonectomies, 16 pleurectomies and 18 palliative procedures as talc pleurodesis or tumour debulking. A total of eight patients only received chemotherapy that was in all cases platinol-based in combination with gemcitabine, mitomycin or others (Table 2).

Survival data were assessed for 129 patients with a median overall survival of 11.7 months (95% CI 9.7; 13.7).

PTEN expression was lost in 62% of the cases (score 0) whereas 14% presented weak (score 1), 10% moderate (score 2) and 14% strong expression of PTEN (Fig. 1). All PTEN expression levels were observed in every histological subtype

Table 2
Univariate analysis of different prognostic factors on survival: PTEN-score (0 vs 1–3), age (≤62 vs >62), surgery (yes vs no), histology (epithelioid, vs biphasic vs sarcomatoid)

	p-Value
PTEN-score	0.0003
Age	0.02
Surgery	0.0015
Histology	0.01

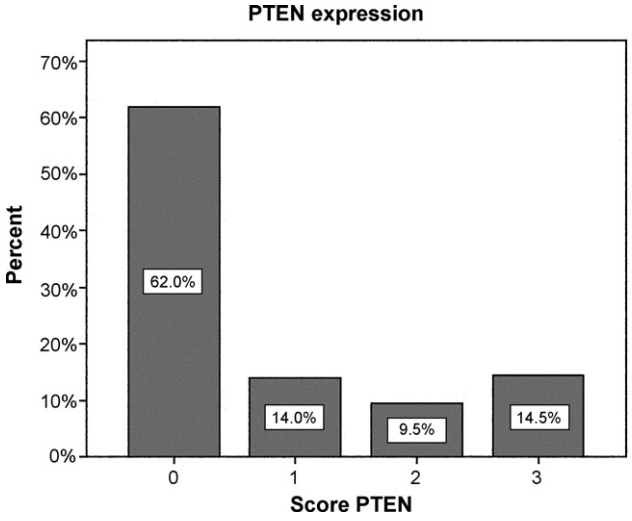

Fig. 1. PTEN expression score 0 (negative), 1 (weak), 2 (moderate) and 3 (strong expression).

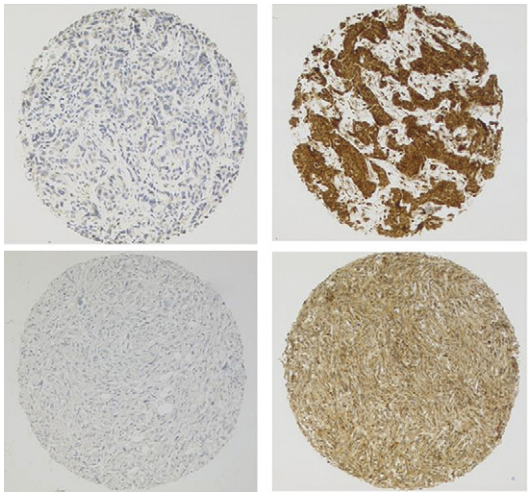

Fig. 2. Malignant mesothelioma tissue microarray: immunohistochemistry with PTEN antibody. No versus strong expression of an epithelioid malignant mesothelioma (upper panel) and no versus strong expression of a sarcomatoid malignant mesothelioma (lower panel).

(Fig. 2) and statistically PTEN expression did not correlate with the histological subtype (gamma-coefficient 0.01, $p = 0.9$).

Survival was strongly correlated to PTEN expression comparing no (score 0: median survival 9.7 months, 95% CI 7.9; 11.7) versus weak—moderate—strong expression (score 1—3: median survival 15.5, 95% CI: 3.8; 27.2) (log rank $p = 0.0001$) (Fig. 3). Even the comparison of the different PTEN expression scores (0 vs 1 vs 2 vs 3) showed a significant impact on overall survival ($p = 0.0003$) (Fig. 4). Moreover, the histological subtype significantly influenced the overall survival with the best survival data of patients with an epithelioid or biphasic subtype (median survival 12.7 months, 95% CI 8.4; 17 and median survival of 13.1 months, 95% CI 10.9; 15.3, respectively) and a shorter survival of patients with a sarcomatoid subtype (median survival 6.5 months, 95% CI 4.9; 8.2) (log rank $p = 0.01$). Patients undergoing surgery had a significant better median survival (14.2, 95% CI 9.45; 18.89) than patients without surgery (9.6, 95% CI 6.9; 12.4) ($p = 0.0015$) and younger patients ($\leq$62) also had a significant better prognosis with a median survival of 12.6 months (95% CI 8.4; 16.9) in comparison to older patients (median survival of 10.9, 95% CI 6.2; 15.6).

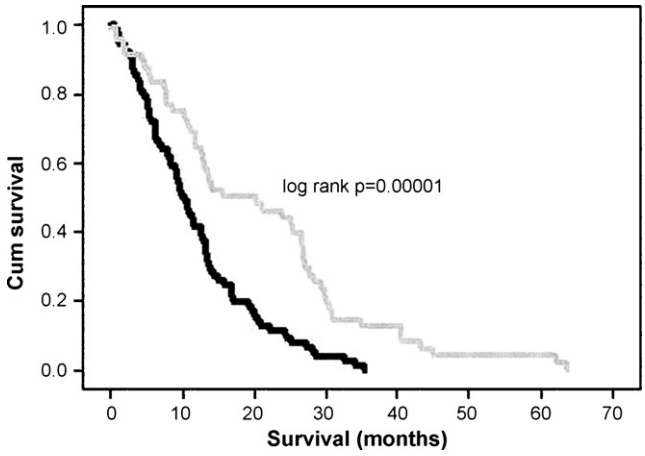

Fig. 3. Survival according to PTEN expression. PTEN negativity (black) versus PTEN expression (weak—moderate—strong expression, grey).

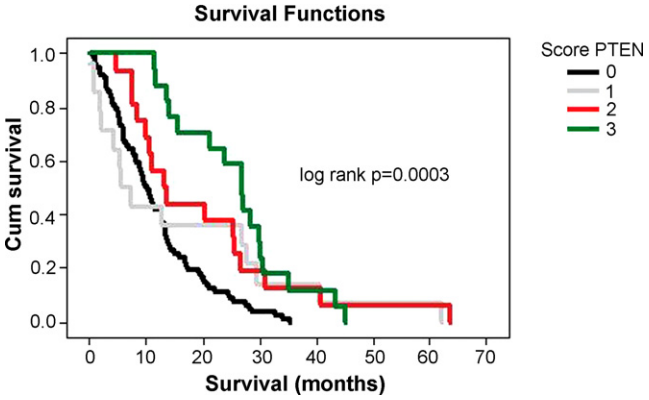

Fig. 4. Survival according to PTEN expression. PTEN negativity (0: black) versus weak (1: light-grey), moderate (2: dotted) and strong (3: dark-grey) PTEN expression.

Cox regression analysis revealed a prognostic influence of PTEN expression (no vs weak—strong expression) on survival ($p = 0.003$) independently from the histological subtype (epithelioid vs non-epithelioid) ($p = 0.7$), age ($p = 0.3$) or surgery (0.09).

4. Discussion

In this large TMA-based study, we were able to demonstrate that loss of PTEN expression occurs in 62% in MPM. Loss of PTEN expression was strongly correlated with shorter survival, independently from the histological subtype. Different biomarkers for the prediction of patient prognosis have been suggested for MPM patients. However, previous immunohistochemical biomarker screening of MPM was based on relatively small numbers of tissue samples [12—15]. In contrast, our study used a TMA with MPM tissue of 341 patients. Clinical data, including staging were available from

206 patients. Complete clinical follow-up data were obtained from 129 patients allowing retrospective survival analysis. Although data assessment was performed retrospectively and therefore not as complete as in prospective analysis and treatment concepts were inconsistent at this time period, the underlying study represents to our knowledge the largest immunohistochemical screening of mesothelioma specimens for the expression of a biomarker with correlation to clinical data. There is only one other report looking at EGFR expression in mesothelioma [16]. Numerous investigations have meanwhile provided strong evidence on the representativeness of TMA data for biomarker analysis and on the reproducibility of the biological relevance of several markers, e.g. the clinical significance of Ki-67 labeling index in bladder cancer or the prognostic role of steroid hormone receptor expression and Her2 neu amplification/overexpression in breast cancer [17].

An association between PTEN negativity by immunohistochemistry and poor prognosis was previously shown for some other cancer types, such as breast and renal cell carcinoma [18,19]. PTEN inactivation is probably the most common [20,21] as well as the most potent lesion of the oncogenic phosphatidylinositol-kinase (PI3K) signalling pathway, which has been implicated in nearly all aspects of tumour biology: cell transformation, growth, proliferation, migration, protection, apoptosis, genomic instability, angiogenesis, metastasis and cancer stem cell maintenance [20,22]. Aberrant PI3K pathway signalling is estimated to be present in >30% of human cancers [20] and occurs beside oncogenic alterations by inactivation of PTEN which is the pathway's most important regulatory brake. It is a plasma-membrane lipid phosphatase that antagonises the function of the PI3K-AKT/protein kinase B (PKB) pathway. One key protein that is dephosphorylated by wild-type PTEN is the serine-threonine kinase AKT. Phosphorylated AKT, the active form of this kinase, promotes cell survival through multiple pathways, many of which are associated with cell growth and survival. Levels of phosphorylated AKT have been reported to be increased in multiple tumours [23]. It was also demonstrated in mesothelioma that the AKT/mTOR pathway is frequently activated in both human and murine MPM specimens and MPM cell lines [24]. Immunohistochemical analysis revealed elevated levels of phospho-AKT in nearly two-thirds of human primary MPM. A strong association with elevated phospho-mTOR positivity in the same tumours confirmed activation of the AKT pathway [8].

PTEN silencing occurs via loss of heterozygosity (LOH) at locus 10q23.3 and mutation of the PTEN tumour suppressor gene in endometrioid and prostate cancer. In MPM, chromosome 10 losses have been detected by comparative genomic hybridisation [9], suggesting PTEN as the target tumour suppressor gene on 10q in MPM. However, a PTEN loss of heterozygosity (LOH) was found in only 1 out of 9 cell lines and protein loss in only 2 out of 26 MPM by Altomare et al. [24]. The discrepancy in the immunohistochemical result can be explained by using different antibodies (Santa Cruz vs Novocastra). According to our experience, the antibody by Novocastra shows the best correlation with LOH of PTEN. Nevertheless, further studies are necessary to evaluate the mutation status of this tumour suppressor gene in MPM. Apart from this study, PTEN expression in primary MPM specimens was not yet analysed in a larger extent. Importantly, Mohiuddin et al. [25] have recently demonstrated in mesothelioma cell lines transfected with adenoviral vector AdPTEN that apoptosis via inhibition of AKT phosphorylation occurs.

In summary, our data strongly indicate that PI3K pathway is involved in MPM carcinogenesis and is an independent prognostic factor. As novel agents like PI3kinase-inhibitors and mTOR-inhibitor, which interfere upstream or downstream to PTEN, are already under clinical investigation, there might be a rationale to use these new low-toxicity pharmaceuticals for MPM.

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Appendix A. Conference discussion

Dr A. Patterson (*St Louis, MO*): I would like to congratulate you with this very elegant presentation and I think it is very interesting. I would like to ask you about the survival of your patients, if there were any patients who were cured of mesothelioma? Because my impression from the survival cases you presented was that there were no cures; that all patients even died early from mesothelioma, but maybe the progression was slower in patients with PTEN factor.

So I think it is very important to state if this factor is prognostically good that the patient can be cured or the progression is slower, that is the question.

Dr Opitz: As mentioned, the biopsy specimens date from the beginning of 1975 until 2004. At this era there was no standardised treatment for malignant pleural mesothelioma and over 70% of the biopsy specimens were extracted during autopsy. The majority of the patients had no treatment or palliative approaches, only a few underwent extrapleural pneumonectomy mainly after 2000. Therefore, these data cannot be correlated with treatment. We perform now a prospective analysis in order to assess treatment response after chemotherapy and prognosis of trimodality therapy.

Dr M. Zielinski (*Zakopane, Poland*): It was a great presentation. I think this kind of work is critically important to understand tumour biology not only for a mesothelioma but also for all malignancy that we deal with. It is great to have 300 cases of pathologic specimens to review retrospectively but as you just said the majority of these patients had really no idea what the clinical staging was. So do you have any sense of what has given you recent practice of detailed staging and surgical resection in patients who you think have favourable disease. Do you think that PTEN influence on survival would actually have an impact in patients who have favourable tumours and undergo resection?

Do you think patients with PTEN expression are much more likely to present with advance disease and patients without PTEN expression are very unlikely to have, in reality, favourable lesions?

Dr Opitz: PTEN was already assessed in other tumours like renal carcinoma or breast carcinoma and it was proven that the stage of disease and the response to therapy correlated to PTEN expression. There are also studies showing that chemotherapy resistance is also dependent on the loss of PTEN expression because of Akt-activation. So it would be very interesting to see if this is also the case for mesothelioma patients.

Dr A. Turna (*Istanbul, Turkey*): You apparently used a tissue bank, but did you look at the heterogeneity of the PTEN expression throughout the tissue, because usually mesothelioma is known to be heterogeneous in terms of oncogenic protein expression. And a second question is that do you know the rate of the patients who are first generation Turkish immigrants among your patients? These patients could be different because of the carcinogenesis under asbestosis.

Dr Opitz: First to your second question: There was no Turkish immigrant in this series. It is true that PTEN is known for many tumours to have a loss of heterozygosity but we just started to look at this. From another study in 18 mesothelioma patients they showed that disarrangement of PTEN seems not to be frequently involved in mesothelioma.

Dr G. Varela (*Salamanca, Spain*): I wonder if you should have measured the interobserver variability because scoring this way is rather subjective.

Dr Opitz: Yes! We should do that, it is true.