Abstract. Background: Pulmonary epithelioid haemangioendothelioma is a rare endothelial tumour without standard treatment. For this reason, our aim is to present contemporary research outlining new therapeutic possibilities; thus in vitro and in vivo methods were combined. Patients and Methods: Pulmonary epithelioid haemangioendothelioma was diagnosed in a 49-year-old female patient. A bronchial excision was obtained from a parenchymal lesion, and the excised sample was manipulated with in vitro-standardized experiments to support the diagnostic and therapeutic procedures. Results: according to in vitro examination of tumour pulmonary and metastases from bone, carboplatin, docetaxel and pharmarubicin was the most efficient treatment modality. Conclusion: Currently, pulmonary epithelioid haemangioendothelioma does not have any standard treatment; the most efficient therapeutic regimen was gradually developed by combining in vitro and in vivo methods, which proved to be an efficient therapeutic modality hitherto.

Pulmonary epithelioid haemangioendothelioma (EHE) is a rare endothelial tumour which is localized mainly to the superficial and deep soft tissues, the bone and the liver (1, 2). The pulmonary form of EHE (PEHE) is classified in the group of intermediate malignancy, but several experts describe it as a malignant tumour (3). Only half of cases have non-specific pulmonary symptoms, such as chest pain, effort dyspnea, cough and sputum (4, 5). The diagnosis of PEHE is difficult and often incidental. The most common radiological features are multiple, uni- or bilateral nodules with a well- or ill-defined border (4, 6). This tumour has no standard treatment. Several chemotherapeutic agents have been tried, mostly carboplatin with etoposide, but chemotherapy appears to have limited effect (2, 4, 7, 8). Our case of a 49-year-old female patient with PEHE, had already developed multiplex bone dissemination at the time of presentation. We were faced with disseminated disease and there was no approved therapeutic protocol due to the rarity of PEHE. Consequently, we were required to research the most efficient therapeutic regimen, combining in vitro and in vivo methods. We initiated an experimental treatment which proved to be an efficient therapeutic modality.

Case Report

The 42-year-old female patient first presented in 2002, due to abnormalities on an annual screening chest X-ray, although at that time she was asymptomatic. A radiological examination [chest X-ray and computed tomography (CT)] detected multiple, bilateral nodules of 5-10 mm in diameter with well-defined margins, which were highly suggestive of multiple metastases. The bronchoscopy examination failed to reveal any abnormalities; therefore, a video-assisted thoracoscopic lung biopsy was obtained. The histological examination revealed there was a sclerotic haemangioma without malignant cells; the lesion was located in the lung parenchyma, and the proliferative activity was low (<3%). The decision was made to observe the patient, but she did not return to our clinic.

Seven years later, she was admitted to our hospital due to cough, weight loss, effort dyspnea, generalized arthralgia and bone pain. Digital clubbing was noticed on physical examination. The chest was examined by X-ray and CT scan. The multiple bilateral nodules identified in 2002 remained constant, while a new, 36 mm in diameter, irregular parenchymal lesion with ill-defined margins on the right upper
lobe and new right hilar and mediastinal lymphadenomegaly had appeared (Figure 1). During bronchoscopy, the right upper lobe bronchus was found to be concentrically narrowed and the segmentation of the right upper lobe was not visible. A bronchial brush biopsy and an excision were taken. The excised sample was divided into two parts: one part was sent for pathological examination and the other part was manipulated with in vitro standardized experiments to support the diagnostic and the therapeutic procedures.

In vitro protocol, cell culture techniques. Cell culture technique: As lung cells are equivalent to modified mesodermal and endodermal cells, a similar methodology to the one used for hypophyseal cell cultures was applied. Details of the cell culturing procedure are described in the literature (11, 12). The bronchoscopy sample was removed under sterile conditions. Healthy and tumorous samples were carefully separated from other tissue under a preparative microscope. The tissue was enzymatically digested with 0.2% trypsin (Sigma, Hamburg, Germany) in phosphate-buffered saline for 20 min, and with 0.17 μg/ml collagenase (Sigma, Hamburg, Germany) for an additional 60 min, and with 1% dispase (Sigma, Hamburg, Germany) at 37°C. The enzymatic dissociation was stopped by the addition of 100 μg/ml trypsin inhibitor (Sigma, Hamburg, Germany), and 20% foetal calf serum (FCS) (4°C). Mechanical dissociation of the tissue was performed on nylon blutex sieves (pore size-48 μm). The cell viability was 98-100%. The dispersed cells were placed into 24-well plastic plates (Nunc, Orlando, FL, USA) coated with 1-100% collagen type IV (Sigma). The starting cell density was $10^5$ cells/ml of Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 20% FCS (Gibco, New York, NY, USA), 1 IU/ml penicillin and 1 IU/ml streptomycin. The 3-D cultures were attached to a collagen (10%)-treated filter (pore size-48 μm). The cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. The culture medium was changed daily. After trypsinization (0.2%, for 30 min.) and trypsin inhibitor treatment, explant cultures were prepared. The normal and tumorous tissues were attached to a plastic petri dish.

Samples of cell cultures were prepared from normal (control: C) and tumourous (Tu) tissues to examine the toxic effects of chemotherapeutic agents. Three in vitro model systems were created: i) from the intact parts of the tissues (which presented low proliferative activity and contact inhibition), referred to as control (C); ii) from the tumourous clone (the cells which lost their ability for monolayer proliferation dimensional orientation and/or contact inhibition; PEHE Tu); and iii) mixed: intact and tumourous cells (PEHE M).

Measurement of total protein content. A modified Lowry Method and a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) were used for the determination of the total protein content (13, 14). Cell viability was measured with trypan blue to determine intact cells (15, 16).

Results of analyses. The pathological analysis revealed undifferentiated, atypical, epitheloid tumour cells, which infiltrated the subepithelial area (Figure 2). The result of diagnostic immunohistochemical examinations showed loss of CD34 and factor VIII antigen, score of CD31:3+, Ki67:1+ (>30%), and negative status for oestrogen and progesteron receptors. The final histological diagnosis was PEHE. Our pathological finding was compared with that of the sample obtained in 2002. The earlier diagnosis was reconsidered, and PEHE was diagnosed, for which the tumour has no standard treatment.

According to data from the literature, carboplatin is the most efficient chemotherapeutic agent against PEHE (8). The monolayer, explant and 3-D cultures (C and Tu) were treated with carboplatin (2.6 ng/ml), with and without the resistance modifier promethazine (Pipolphen) (2.5 μg/ml).
It was detected in vitro that the mean (±SEM) protein production (212.14±7.11 μg protein vs. 408.48±36.13 μg protein, n=5). Carboplatin was added to the PEHE Tu clone and the proliferative activity declined (212.14±7.11 μg protein vs. 301.59±17.96 μg protein, n=5). The reduction was more definitive when promethazine was added to the PEHE Tu clone with carboplatin (301.59±17.96 μg protein vs. 259.88±12.17 μg protein, n=5).

Our in vitro examination showed carboplatin to be effective, hence first-line chemotherapy treatment was initiated. Our patient underwent a 6-cycle carboplatin [5AUC]-etoposide (100 mg/m²) regimen. The radiological findings (chest CT) following chemotherapy showed partial regression. Significantly (20%) reduced proliferative activity was identified in the repeated bronchoscopic biopsy samples. The tumour cells exhibited intense apoptosis; tumour clones did not grow in culture and only control and mixed cell cultures exhibited proliferation (Figure 3a and b). These subsequent cultures were treated with different cytotoxic agents after first-line chemotherapy (Figure 4a). Docetaxel proved to be the most efficient agent in the in vitro assay.

Progression was detected within four months and second-line docetaxel treatment was started. Four cycles of docetaxel [75 mg/m²] treatment resulted in partial regression.

After the start of first line chemotherapy, the patient complained of multiplex bone pain, and multiplex bone metastases were detected. The first- and second-line chemotherapy treatments were effective with respect to the pulmonary disease, but collaterally, the bone disease was progressive. Due to severe pain, we decided for operation of the bone lesion; the histological analysis from the operative resection revealed metastasis of PEHE. The proliferative activity was high and diverse (80%). According to the in vitro examination of metastases from bone, pharmorubicin was the most efficient treatment modality (Figure 4b). The patient was regularly observed, and progressive, multiplex, bulky 18F-fluorodeoxyglucose cumulative lymphadenomegaly was detected three months after the end of second-line chemotherapy by positron emission tomography/CT. Due to the extensive lymphadenomegaly, third-line treatment with pharmorubicin (80 mg/m²) was initiated which resulted in stable disease at the end of the cycle.

Discussion

EHE usually affects young and middle-aged patients, with a female predominance (male:female 1:2-4) (1, 4). Patients range in age from 7 to 83 years, with a median onset of 36 years (5, 17, 18).

EHE was originally described by Dail and Liebow in 1975 (19). This was believed to be an aggressive form of bronchoalveolar cell carcinoma that invaded adjacent blood vessels; hence the name intravascular bronchioalveolar tumour was originally given (4, 19).

Almost half of the cases are asymptomatic; EHE is often incidentally diagnosed or associated with only minor non-specific pulmonary symptoms, such as chest pain, effort dyspnea, cough and sputum (4, 5). Multiple uni- or bilateral...
nodules with well or ill-defined margins are the most common feature of PEHE on chest radiography (4, 6). The dissemination of PEHE outside the pulmonary parenchyma is rare. To our knowledge only three patients are reported to have suffered from extensive bone metastases (7). PEHE is positioned between haemangioma and conventional angiosarcoma. It is classified in the group of tumours as intermediate malignancy, but there have been proposals to reclassify it as a malignant tumour (3). The prognosis of patients with PEHE is unpredictable, with life expectancy ranging from 1-15 years (2).

In our case, we were presented with disseminated disease and there was no approved therapeutic protocol due to the rarity of PEHE. We researched the most efficient therapeutic regimen combining in vitro and in vivo methods. Our experimental study of biopsy-sourced cell cultures treatments lead to initiating which proved to provide a new efficient therapeutic modality.

Conflicts of Interest

The Authors declare no conflicts of interest.

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