# Malignant Fibrous Histiocytoma of the Parotid Gland

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## Summary

**We described a rare malignant fibrous histiocytoma of the parotid gland (MFH) in a 63-year-old woman. During six months the tumour size became 10 cm in diameter with skin ulceration. The tumour was examined morphologically, by immunohistochemistry and molecular biology methods – FASAY and CGH. The histology revealed a storiform-pleomorphic type of MFH with high mitotic rate. The FASAY method identified a non-mutated p53 gene. The chromosomal changes were identified by the CGH method and 6 cytogenetic changes were found in the tumour cells (deletions at 8p12-p22, 13q32-qter, 14q24-qter, and gains of chromosomal material at 5p, 8q12-q23, and Xq25-qter). The patient died shortly after the beginning of chemotherapy. Autopsy revealed brain and cerebellar haemorrhage. No other tumour foci were proved. In view of short course of disease we lack the data about the influence of the non-mutated p53 gene on the prognosis and therapy.**

**Key words: malignant fibrous histiocytoma - parotid gland – immunohistochemistry - molecular biology methods - p53 gene - comparative genomic hybridization**

## Souhrn
### Maligní fibrózní histiocytom příušní slinné žlázy

**Autoři popisují vzácný výskyt maligního fibrózního histiocytomu (MFH) parotis u 63leté ženy. Během šesti měsíců dosáhl nádor velikosti 10 cm a kůže nad ním zvředovatěla. Nádor byl vyšetřen morfologicky, imunohistologicky a metodami molekulární biologie- FASAY a CGH. Histologický nález ukázal storiformně-pleomorfní typ MFH s vysokým mitotickým indexem. Pomocí metody FASAY byl zjištěn nemutovaný gen p53. Metodou CGH jsme identifikovali chromozomové změny v karyotypu nádorových buněk. Prokázalo se šest chromozomových změn (delece úseků 8p12-p22, 13q32-qter, 14q24-qter, zisky oblastí 5p, 8q12-q23, a Xq25-qter).**
**Pacientka krátce po zahájení chemoterapie zemřela. Při pitvě se nalezlo rozsáhlé krvácení do mozkové a mozečkové hemisféry. Další nádorová ložiska se pitvou neprokázala. Vzhledem k relativně krátkému průběhu onemocnění nebyla možnost stanovit vliv nemutovaného genu p53 na prognózu onemocnění.**

**Klíčová slova: maligní fibrózní histiocytom – příušní slinná žláza – imunohistologie – metody molekulární biologie - gen p53 – komparativní genová hybridizace**

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Malignant fibrous histiocytoma (MFH) is common in deep soft tissues of late adult life, with rare occurrence in the head and the neck. Almost 30 cases of MFH in the parotid gland have been reported so far (5, 9, 14). In this report we document an additional case of MFH in the right parotid gland with application of molecular pathology methods. We used the FASAY (Functional Analysis of Separated Alleles in Yeast) method which enables identification of mutated or non-mutated p53 gene, and the CGH (Comparative Genomic Hybridisation) method for identification of chromosomal alterations in tumour cells.

## Case report

A 63-year-old woman was admitted with nodular resistance in the region of the right parotid gland. During six months the tumour continually enlarged to 10 cm in diameter and skin above the tumour ulcerated. The patient was admitted to hospital. A poorly defined swelling in the region of the right parotid gland (Fig.1) was revealed. A non-encapsulated tumour surrounding vessels and peripheral nerves was found and surgery could not be performed. The tumour totally destroyed the gland. Histologically, it consisted of spindle cells and

scattered large multinucleated cells. A brisk mitotic rate was present. The diagnosis was not unequivocal and different types of tumours were considered: osteoclast-type giant cell tumour, MFH, dedifferentiated carcinoma with osteoclast-like giant cells. After the beginning of chemotherapy the patient's condition deteriorated and she died of acute haemorrhage to the left cerebral and cerebellar hemisphere.

Autopsy proved a primary tumour in the right parotid gland. No other tumour and/or metastases were found. In the brain and cerebellum significant haemorrhage was found. The histology of the parotid gland tumour was variable. In some regions fascicles of spindle cells were arranged in a storiform pattern (Fig. 2). The nuclei were elongated and hyperchromatic. A more pleomorphic pattern was found in other regions. Plump spindle cells with hyperchromatic elongated or oval nuclei were arranged in short fascicles and a large number of giant cells with bizarre nuclei were predominant in the lesion (Fig. 3). Also, mitotic activity was high and more prominent in these pleomorphic areas. Atypical mitotic figures were found. A moderate number of small lymphocytes and plasma cells were scattered in the stroma. In some regions irregular areas of necrotic tumour tissue were present with granulocytes around. An MFH storiform-pleomorphic type was diagnosed.

## Material and methods

For histological examination, the tissue samples were fixed in 10 % buffered formalin and embedded in paraffin. The slides were stained with hematoxylin and eosin.

For the immunohistochemistry, 5 μm thick sections from paraffin blocks were deparaffinised in xylene, hydrated in decreasing concentrations of alcohol solution and washed in Tris buffer prior to staining. Immunohistochemistry was performed using the avidin-biotin-peroxidase complex method.

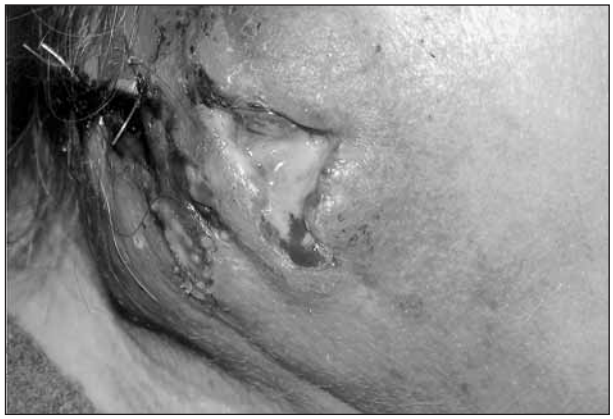

**Fig. 1. Ulcerated tumour of the right parotid gland**

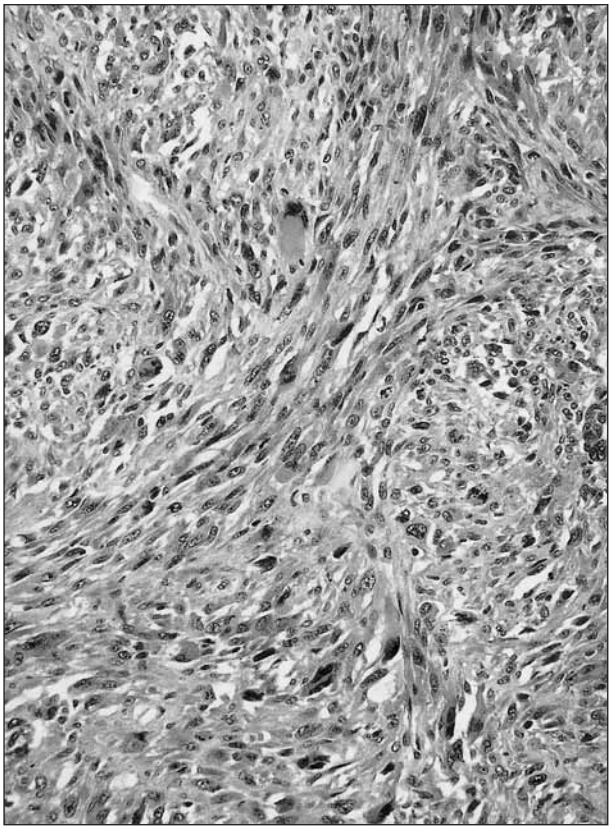

**Fig. 2. Malignant fibrous histiocytoma, storiform-pleomorphic type with a predominantly storiform pattern. Hematoxylin-eosin stain, magnification 200**

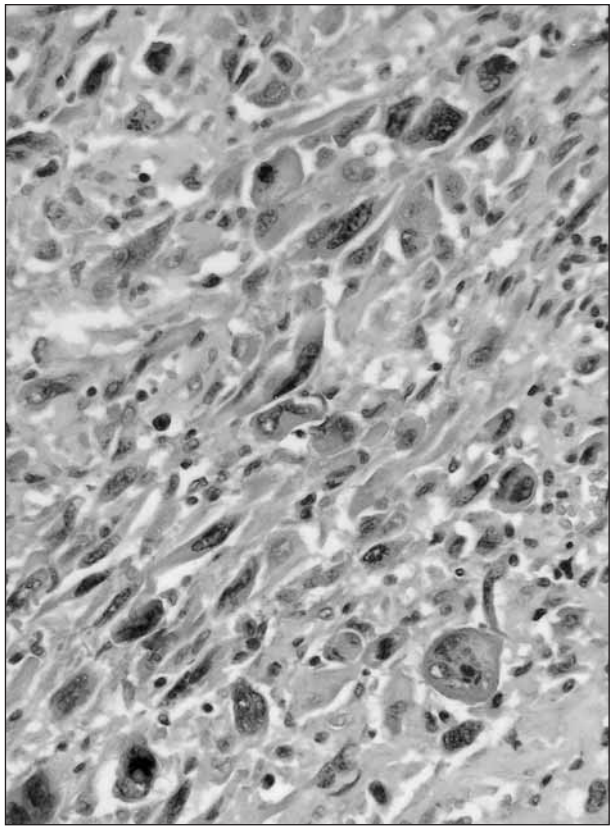

**Fig. 3. Pleomorphic pattern of malignant fibrous histiocytoma, storiform-pleomorphic type. Hematoxylin-eosin stain, magnification 450**

The antibodies and sources used in this report are listed in Table 1.

## Methods of molecular biology

### Functional Analysis of Separated Alleles in Yeast (FASAY)

FASAY was performed using the protocol described by Flaman et al. (3), with the use of primers P3 (5'-CCT-TGC-CGT-CCC-AAG-CAA-TGG-ATG-AT-3'), complementary to nucleotides 101-126 of the p53 gene, P4 (5'-ACC-CTT-TTT-GGA-CTT-CAG-GTG-GCT-GGA-GT-3'), complementary to nucleotides 1094-1122 of the p53 gene, Pfu DNA Polymerace (Stratagene). Yeast cells were co-transformed with the PCR product, the linearised vector pSS16, and the salmon sperm DNA carrier (GibcoBRL) by the lithium acetate procedure, as described by Ishioka et al. (7). Transformed yeast cells were plated on minimal medium without leucine and with a low amount of adenine (5 ug/ml), followed by incubation for 2–3 days at 35 ºC and then for 2–3 days at room temperature.

### Comparative Genomic Hybridization (CGH)

CGH was performed as previously described by Kallioniemi et al. (8). Briefly, tumour DNA from native frozen tissue was prepared using chloroform extraction and labelled with SpectrumGreen-dUTP using Nick Translation Kit (Abbott-Vysis, Inc., Downers Grove, IL, USA). Simultaneously, normal human genomic DNA from peripheral blood lymphocytes was labelled with SpectrumRed-dUTP. The labelled DNA samples were then used as a probe for *in situ* hybridization to normal metaphase chromosomes.

Fluorescent signals were captured using a CCD camera COHU 9410. The chromosomes were karyotyped and ratio profiles calculated using the LUCIA 4.80-CGH System for Image Processing and Analysis (Laboratory Imaging, Ltd., Prague)

## Results

The tumour infiltrated the whole parotid gland and the surrounding tissues. The histological appearance and immunophenotype of the present case were consistent with the diagnosis of storiform-pleomorphic MFH. In the immunohistochemistry, positive findings were present in mononuclear tumour cells with antibodies CD68(KP1), alpha1 antitrypsin, alpha1 antichymotrypsin. Some multinucleated cells were also CD68(KP1) positive. The immunohistochemistry failed to detect any markers of other malignant tumour types.

**Tab. 1. The antibodies and results**

| Marker | Mononuclear cells | Multinucleated cells |
|---|---|---|
| CD68 (KP1) | +[1] | +[1] |
| EMA | - | - |
| AE1-AE3 | - | - |
| SMA | - | - |
| S100 protein | - | - |
| Vi | - | - |
| CD34 | - | - |
| A1AT | +[1] | - |
| A1ACT | +[1] | - |
| Lysosome | - | - |
| Ki-67 | 30–40 % | |

Explanation: SMA – smooth muscle actin
Vi – vimentin
A1AT – alpha 1 antitrypsin
A1ACT – alpha 1 antichymotrypsin
[1] – some cells

In order to characterise general changes present in the tumour cells, we performed p53 status analysis and CGH. The p53 status analysis of the tumour cells by FASAY revealed a wild p53 gene. A total of 6 cytogenetic changes at 5 different chromosomes were found in the tumour by CGH. These included regional deletions at 8p12-p22, 13q32-qter, 14q24-qter, and gains of chromosomal material at 5p, 8q12-q23, and Xq25-qter.

## Discussion

MFH has a predilection for the extremities and retroperitoneal space, thus the incidence in the head and the neck is low. Only 3-10 % of all MFHs is recorded in this location. Primary MFH of the parotid gland is rare (1, 2, 9, 17, 18). We described a locally destructive growth of MFH in a 63-year-old woman.

Based on the histological pattern, four types of MFH have been recognized: the most common storiform-pleomorphic type; the myxoid type; and the less common giant-cell and inflammatory types (18). Other types of above mentioned tumours, which should be taken into consideration, were excluded by morphology and immunohistochemistry. However, the role of immunohistochemistry in the diagnosis of MFH is traditionally an ancillary one (4,18). The tumour penetrated into the neighbourhood of the gland and infiltrated soft tissues. Surgery could not be performed.

The prognosis of MFH is generally poor. Kariya

et al. (9) reported 23 cases of parotid MFH from the literature. Five patients died as a direct result of their tumours and, in one case, local recurrence after 6 years was found. In the other cases only short or none clinical follow-up proceeded. Only four patients had clinical follow-ups for more than one year and were without disease.

The examination for p53 gene was performed. The data suggest that the p53 gene plays an important role not only in the tumorigenesis but also in therapy response. Mutations in the p53 gene have been associated with poor prognosis in a large number of neoplasms (12). Soft tissue sarcomas frequently carry p53 mutations reducing chemotherapeutical response (16). Previous studies used polymerase chain reaction (PCR) and sequence analysis or PCR single-strand conformation polymorphism for detection of the p53 gene. The results indicated worse prognosis and a shorter overall survival in patients with alterations in the p53 gene (10,12,13). In this case we examined the status of the p53 gene by the FASAY method and detected a non-mutated p53 gene. The patient died early at the beginning of chemotherapy. The significance of the wild p53 gene for therapy in this case remained unclear.

CGH allows analysis of DNA sequence copy number differences in a one-step global screening procedure (8). In this study, unbalanced chromosomal changes were determined in the karyotype of the MFH patient tumour tissue (3 losses and 3 gains). Specific chromosomal region abnormalities in MFH are documented in the literature. These abnormalities can indicate the degree of tumour progression and genetic instability; some genes connected with tumorigenesis have been localized in these regions (15, 20). The most frequent abnormalities found in MFH are gains of genetic material on chromosomal arms 1p, 9q, 5p, 7p, 7q, 13q, 17q, 19q, 20q, and losses at 1q, 2q, 3p, 4q, 11q, and 13q regions (6, 11, 19). Mainly the excess of genetic material at 7q32 has a significant prognostic meaning because its occurrence correlates with the decrease of the patient's survival. On the other hand, gain of 17q in MFH is associated with a longer disease-free survival and a low risk of developing metastasis (19).

In agreement with the data (6, 11, 19), certain documented chromosomal abnormalities (gain of 5p, loss of 13q) were also detected in our case. In myxoid and storiform-pleiomorfic subtype of MFH gain of 5p and loss of 13q did not have any prognostic value (11). However, there are no specific data about cytogenetic abnormalities in MFH of the parotid gland, therefore we are unable to determine the prognostic value of these abnormalities.

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