Introduction

Central nervous system (CNS) metastases of neuroblastoma, although uncommon, are now being increasingly reported in long-term survivors [1]. However, cerebral recurrence more than 5 years after diagnosis is extremely rare [2, 3]. We herein present the case of a young woman diagnosed initially with a stage IV spinal neuroblastoma as an infant, suffering from a CNS tumor as well as hepatic and peritoneal metastases 22 years following complete remission achieved by myeloablative chemotherapy. The CNS malignancy challengingly referred to the differential diagnostic dilemma of a late relapse of neuroblastoma versus secondary supratentorial primitive neuroectodermal tumor (sPNET). The histopathological findings as well as molecular genetic analyses of the primary and recurrent tumors are discussed with particular reference to prognostic markers for late relapse in neuroblastoma versus secondary sPNET.

Clinical Case Illustration

22 years ago, at the age of 12 months, the female patient developed a paraparesis and underwent hemilaminectomies D9–12 and microsurgical resection of a spinal neuroblastoma (fig. 1a). Tumor staging revealed stage IV disease with evidence of metastases to the right zygomatic bone, parotid gland, as well as in supravacular and axillary lymph nodes. Postoperatively, she ex-
experienced a full neurological recovery and received chemotherapy with 2 × DTIC (dimethyl-trizeno-imidazole-carboxamide, 230 mg), vincristine (0.5 mg), endoxan (40 mg) and adriblastin (7.7 mg). When the metastatic lesions were found to be progressing, the regimen was changed to 8 × paltinex (45 mg) and VM26 (teniposide, 37 mg).

After 20 years in complete remission, the patient developed headache, diplopia and blurred vision. Cranial magnetic resonance imaging revealed a solid, 2.5 × 2.5 cm sized, inhomogeneously enhancing right temporopolar tumor, infiltrating the dura and the adjacent superior orbital fissure. There was no more than a sliver of perifocal edema (fig. 2a), and meningioma was suspected. With the exception of a marked thoracolumbar scoliosis, most probably due to the combined effects of multisegment hemilaminectomy followed by irradiation of the entire spine at infant age, spinal magnetic resonance imaging was without pathological findings. The patient underwent an osteoplastic right temporal craniotomy when the tumor could largely be resected, leaving a small marginal gadolinium-enhancing residuum at the apex of the right orbit. Histomorphologically, a malignant small blue round cell tumor was found (fig. 1b). Immunohistochemically, tumor cells showed focal positivity for vimentin and neural cell adhesion molecule (NCAM), but were negative for neuron-specific enolase (NSE), glial fibrillary acid protein (GFAP), neurofilament and chromogranin. Neuropathological differential diagnoses included sPNET and metastasis of neuroblastoma. Because of lack of urinary excretion of vanillylmandelic and homovanillylmandelic acids and equivocal immunohistochemistry, treatment according to the HIT2000 protocol [4] for PNET was administered (2 × vincristine as well as irradiation of the whole cranium and spine (35.2 Gy with 19.8 Gy boost)). Follow-up imaging 2 months later showed no residual pathological gadolinium enhancement, indicating complete regression of the postsurgical intracranial tumor remnant (fig. 2b).

At the age of 23 years, however, hypodense lesions of up to 5 cm² were detected in the hepatic segments V and VIII on contrast medium-enhanced computed tomography. Biopsy revealed a malignant small round cell tumor with histologic similarities to the initial right temporopolar lesion. Treatment was therefore given with second-line polychemotherapy following the HIT2000 protocol. Irrespective of repeated partial hepatectomies to resect tumor deposits, ascites due to peritoneal involvement developed.

At the age of 25, the patient rapidly developed clinical signs of raised intracranial pressure. A right frontotemporal tumor relapse was found, now diffusely infiltrating the adjacent cerebral hemisphere (fig. 2c). Partial tumor resection was performed, again confirmed as small blue round cell tumor (fig. 1c). This now proved immunohistochemically positive for GFAP, NCAM as well as NSE while showing neuronal structures. 15 days following this intervention and 24 years after initial stage IV neuroblastoma, the patient deceased due to hepatic failure.

Molecular and Cytogenetic Analysis

DNA was isolated from formalin-fixed and paraffin-embedded specimens of the initial spinal as well as from the subsequent cerebral tumor resections. RNA was isolated from fresh-frozen specimens of the cerebral lesions only. No PNET-specific translocations like t(11;22) (q24;q12) could be shown in either of the CNS tumors using RT-PCR. Fluorescence in situ hybridization (FISH) using D2Z (for the centromere of chromosome 2) and N-myc (chromosome region 2p23–p24) was found to be negative for MYCN amplification in primary and both
cerebral tumors. By using comparative genomic hybridization (CGH) both CNS malignancies were analyzed [5], providing comprehensive overview of chromosomal gains and losses (fig. 3a, b). Unfortunately, CGH analyses of the primary neuroblastoma failed due to lower DNA content. Conventional cytogenetic analysis [6] in the CNS tumors not only verified the clonal loss of chromosomes 9 and 15 as already shown by CGH, but in conjunction with multicolor FISH (MFISH) analysis [7] additionally determined the exact hypotetraploid karyotype of the second brain recurrence with a reciprocal translocation between chromosome arms 6q and 11q, t(6;11)(q25;q13), as well as two unbalanced translocations, der(9)t(9;22)(q34;q11) and der(17)t(11;17)(p11;p13) (fig. 3c–e).

**Discussion**

Our case offers peculiarities of prognostic as well as of diagnostic aspects. Of prognostic interest, a clinical course with a 22-year interval between complete remission after myeloablative chemotherapy of an initially stage IV infantile spinal neuroblastoma and secondary fatal CNS tumor is exceedingly rare. The second interesting aspect is the histomorphological and cytogenetic differential diagnosis of the brain tumors, representing either a very late relapse of neuroblastoma or a secondary sPNET.

The median time from initial therapy to CNS recurrence of neuroblastoma is fairly constant, ranging from 13 to 20 months after primary diagnosis, and has a 3-year incidence of between 2.2 and 8.0% [1, 3, 8]. Especially in stage IV disease, tumor frequently recurs even after complete response to initial therapy [1, 9, 10]. Patients with classic stage IV neuroblastoma do, however, very rarely survive long term [2, 11]. Reports of survivals, remission intervals of more than 14 years [11], of refractory recurrent disease [12] and of progressive disease [13] do nonetheless exist. Specifically, however, we could identify only one study [14] which reported a patient who survived for 26 years following the diagnosis of primary multifocal neuroblastoma. On the other hand, secondary sPNETs have been reported in 18 patients to date, most often following treatment with cranial irradiation and intracranial methotrexate therapy for leukemia and lymphoma [15–19], retinoblastoma [20], or astrocytoma [21–23]. To our knowledge, a secondary sPNET following chemo-
Fig. 3. CGH. a First CNS relapse with +7, −4, −9, −10, −15q. b Second CNS relapse with +1p11p31, +8q, −6p21p ter, −9q32qter, −15q, −16p, −17p. c Karyotype of second CNS relapse is hypotetraploid: 77–89, <4n>, XXXX, −4, −5, t(6;11)(q25;q13)×2, −9, −9, t(9;22)(q23;q11)×2, −11, −12, −15, −15, t(11;11)(p13:p13)×2, −18, −19, −22, +1t−6mar. d, e Chromosomes representing the balanced translocation t(6;11) as well as the unbalanced translocations der(9)t(9;22), and der(17)t(11;17) are highlighted along with their MFISH classification colors (with numbers indicating chromosomal origin of material).
therapy and irradiation for neuroblastoma has never been reported before.

The histomorphological and genetical differential diagnostic considerations between late CNS relapse of a neuroblastoma and secondary sPNET require discussion [24, 25]. In molecular genetic terms, neuroblastoma patients can be divided into different prognostic subgroups. One end of the spectrum represents patients under the age of 18 months [26] who carry a favorable prognosis (stages I, II and IVS) and a triploid karyotype [27, 28]. In contrast, at the other end of the spectrum are patients older than 18 months with unfavorable outcome (stages III and IV), a pseudodiploid or near tetraploid karyotype as well as amplification of N-myc oncogene [27, 29]. Our patient suffering from initially stage IV disease displayed favorable risk factors including an age of less than 18 months and non-amplified N-myc oncogene, as retrospectively analyzed.

CGH analyses of 7 CNS relapses of neuroblastoma showed gains at 2p24 (MYCN amplification) in 5 patients together with deletion at 1p, and gains at 17q [3]. Of these 5 patients, 2 additionally showed –11q23 to 25. One patient who had non-amplified MYCN had normal 1p but had +17q and –11q as well as –3p21 to 25, +6p, –15, and +18. For secondary sPNET, no cytogenetic analyses exist. In childhood primary sPNET, distal chromosome 4q loss is the most frequent change which is observed in up to 50% of the sPNET [25]. Other chromosomal regions observed at significant frequencies include losses at 9p, 13q, and 14q [25]. CGH analysis of the first frontotemporal relapse in our patient showed gain of chromosome 7 as well as losses of chromosomes 4, 9, 10p and 15q which are recognized as sequential aberrations occurring during neuroblastoma progression [30], but can in part be seen in primary sPNET [25]. The second cerebral tumor in our patient featured repeated losses of chromosomes 9 and 15, compatible with a clonal origin of both relapses. Conventional cytogenetic analysis in combination with MFISH has revealed net loss of 11q and 17p in the second CNS relapse of our case. Remarkably, net loss of 11q is frequently seen in high-risk neuroblastomas associated with poor prognosis [28, 31–33]. On the other hand, net loss of 11q in combination with a lack of MYCN amplification or 1p deletion, as also present in our case, has been associated with a genetic subgroup of advanced neuroblastomas and is presumably associated with slowly growing tumors which allow the accumulation of secondary aberrations in the longer interval [34]. Compatible with these findings, the survival of 53 months following the first cerebral relapse in our patient also seems extraordinarily long, considering the otherwise uniformly fatal outcomes, usually within 7 months following detection of CNS relapse. Moreover, allelotyping studies have disclosed loss of chromosome 17p in only one of 36 primary sPNETs and one sPNET cell line [25]. The breakpoint at 17p13 in our case might have impaired p53 function. Although p53 mutations are rare in neuroblastomas at diagnosis, mutant p53 has been reported in 4 out of 5 neuroblastoma cell lines, all of which were obtained from tumor after cytotoxic therapy for progressive disease or relapse [35].

Systemic filiarization to the liver in our patient also hints at a late recurrence of the initial neuroblastoma rather than an extracranial manifestation of an sPNET. Extracranial metastases of sPNET are very rare, with only two reports found in the literature [36, 37], one stating cervical lymph node metastases [37], the other vertebral bone marrow and lung metastases [36]. In contrast, liver metastases in neuroblastoma are relatively common, and are seen in 30% of patients with stage IV disease [8].

The importance of complete tumor resection of both cerebral neuroblastoma and sPNET has been emphasized in numerous reports [38–41]. Based on aggressive clinical features, sPNET receives therapies designed for metastatic, high-risk medulloblastomas, which involves more intensive chemotherapy and higher doses of radiation to the head and spine as proposed in the HIT2000 protocol [4]. For CNS relapse of neuroblastoma, surgery and cranial or craniospinal radiotherapy rarely resulted in survival beyond a few months [1, 42]; an exception was reported by Kellie et al. [10] who presented a patient who underwent surgical resection for a CNS parenchymal lesion and was treated with craniospinal radiotherapy, resulting in prolonged remission (>62 months). When additional chemotherapy was given, most investigators chose an etoposide-based regimen with either ifosfamide, cisplatin or carboplatin [1]. Still, remission periods were temporary. The median survivals from the time of CNS recurrence were 1 month up to, at best, 14 months [1].

In summary, we present the very unusual case of a young woman suffering from a brain tumor as well as hepatic and peritoneal metastases 22 years after a stage IV spinal neuroblastoma as an infant, demonstrating the difficulties of differentiating late neuroblastoma relapse from secondary sPNET. Lacking specific immunohistochemical features, the first cerebral tumor at the age of 21 was regarded as sPNET, and we pursued a therapeutic approach consisting of neurosurgical resection as well as irradiation and high-dose alkylator-based chemotherapy
according to the HIT2000 protocol. Two years later the patient suffered from a diffusely infiltrating local recurrence, changing its imaging appearance as well as its immunohistochemical characteristics, now revealing disseminated positivity for NSE and NCAM. Moreover, independent of the change in immunohistochemical characteristics from the first to the second cranial metastasis, which could also be interpreted as a change induced by chemotherapy and irradiation [43, 44], the diagnostic importance of systemic metastases and chromosomal characteristics of neuroblastoma as well as the lack of PNET-specific translocations (EWS/FLI1 gene fusion) in both brain tumors led us to the final diagnosis of a very late relapse 22 years after initial stage IV spinal neuroblastoma.

References


