Expression of Insulin-like Growth-Factor-1 Receptor (IGF-1R) in Peripheral Nerve Sheath Tumors in Neurofibromatosis Type 1

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Abstract. Background: Neurofibromatosis type 1 (NF1) is an autosomal-dominant inherited disease, characterised by the development of nerve sheath tumors. NF1 is the most frequently inherited disease associated with a predisposition for cancer (in particular malignant peripheral nerve sheath tumors: MPNST). NF1 is a progressive disease with phase-like growth spurts of dermal or plexiform neurofibroma (PNF). These tumors can cause severe disfigurement of patients. Growth control of these tumors is poorly understood. The aim of this study was to identify the expression of insulin-like growth factor 1-receptor (IGF-1R) in peripheral nerve sheath tumors. Factor and receptor are involved in the growth control of numerous physiological and pathological processes, including Schwann cell development. Materials and Methods: The investigation included tumors of NF1-patients only (neurofibroma, MPNST). Sections of the specimens were immunohistochemically typed for several antigens (target antigens: IGF-1R, S-100, EMA, CD34, MIB-1), using both single and double-staining methods. Double-staining allowed the sub-typing of the IGF-1R-expressing cells in the mixed nerve sheath tumors. The expression was also investigated in Schwann cell cultures and co-cultures with fibroblasts. Results: Staining of S-100 and IGF-1R, PNF were more intensely marked than MPNST (r=-0.439, p<0.002, N=49). The proliferation index was tumor-type dependent: MPNST>neurofibroma. The IGF-1R-expression correlated positively with the MIB-1 index in neurofibroma (r=0.372, p=0.021, N=38). The receptor expression was higher in PNF than in dermal neurofibroma (r=0.335, p=0.040, N=38). IGF-1R was detected in Schwann cells (S-100 positive) and in perineurial cells (EMA-positive) of all nerve sheath tumors. However, the receptor was also identified in CD34-marked endothelia of MPNST. In Schwann cell cultures, a strong receptor-expression became evident. This expression was independent of co-cultivation of tumor cells with fibroblasts. The statistical calculations excluded the impact of gender on the receptor expression. Conclusion: This investigation provides evidence for the expression of IGF-1R in nerve sheath tumors in NF1. The expression pattern varied between the tumor types, the cell types, and between tumors of the same type. IGF and IGF-1R are a prerequisite to maintain Schwann cell stability in the postnatal period and to prevent Schwann cell apoptosis. The first evidence for IGF-1R expression in mutated Schwann cells may indicate a tumor-type associated receptor expression in NF1.

Neurofibromatosis type 1 (NF1) is an autosomal-dominant inherited disease, affecting about 1 in 3000 individuals alive at birth (1). Neurofibromas, i.e. benign tumors of peripheral nerve sheath cells, are the hallmark of the disease. NF1 is a progressive disease with phase-like growth spurts of dermal or plexiform neurofibromas (PNF). These tumors can cause severe disfigurement of patients. Moreover, NF1 is the most frequently inherited disease associated with a predisposition for cancer, in particular malignant peripheral nerve sheath tumors (MPNST). Plexiform neurofibromas are judged as a precancerous lesion in NF1 patients that can give rise to the development of MPNST. Growth control of neurofibromas in NF1 is poorly understood. The aim of this study was to identify the expression of insulin-like growth factor receptor (IGF-1R) in peripheral nerve sheath tumors. Factor and receptor are involved in the growth control of numerous physiological and pathological processes, including Schwann cell development and regeneration (2-5). Signal pathways via the IGF-1R play a crucial role in the development and progression of malignant diseases (6). At present, no immunohistochemical studies have been published on IGF-1R in nerve sheath tumors in NF1.

Materials and Methods

The investigation included 210 tumors of 68 NF1 patients. All patients fulfilled the updated diagnostic criteria for NF1 as recommended by the US National Institutes of Health (7). All tissues were formalin-fixed in the operating theatre, stored and...
embedded in paraffin. Diagnosis of tumor type was established on adequate sections of HE-stained slices. Sections of the specimens were immunohistochemically typed for several antigens (target antigens: IGF-1R, S-100, EMA, CD34, MIB-1), using both single and double-staining methods. Double-staining allowed the subtyping of the IGF-1R-expressing cells in the nerve sheath tumors. The expression was also detected in Schwann cell cultures and co-cultures with fibroblasts.

Double-staining with anti-IGF-1R and antibodies with different specificity was performed in order to subtype cells expression for IGF-1R. S-100 protein was used to identify Schwann cells (8), epithelial membrane antigen for perineurial cells, and CD34 antigen for endothelial cells (9, 10). EMA antibody was used to demonstrate perineurial cells (11). In contrast to Schwann cells, the perineurial cells are not reactive to anti-S-100 antibodies, nor do Schwann cells react with anti-EMA antibody.

MIB-1 was used to identify proliferating cells. MIB-1 positive cells were counted and a proliferation index (PI) was calculated, as described elsewhere in detail (13). Slices of the tumor specimens were used in all experiments as negative controls that were subjected to the same staining protocol but omitting the primary antibody.

Results

**Immunoreactivity for anti-IGF-1R (Figure 1 and 2).** Out of 210 tumors, 35 (17%) showed no labelling with IGF-1R antibody. The immunoreactivity was weak in a further 90 cases (43%), medium in 63 (30%) and strong in 18 (9%). Tissues of four cases were excluded due to inadequate tissue composition. Focusing on neurofibroma of the plexiform or dermal type, the immunoreactivity for the anti-IGF-1R was demonstrated in EMA-positive perineurial cells. The intensity was strong in 17 cases (9%), medium in 59 (31%), weak in 84 (44%) and absent in 28 (14.5%).

**Double-staining with anti IGF-1R and S-100 (Figure 3).** Six tumors were selected for S-100/IGF-1R staining (4 neurofibroma, 2 MPNST). All tissues showed strong immunoreactivity for IGF-1R. Three of 4 neurofibromas were stained with both antibodies. One of the MPNST was negative for S-100, the second showed S-100-expression.

**Double-staining with anti IGF-1R and anti human epithelial membrane antigen.** Ten tumors were selected for double staining for IGF-1R and EMA. All tumors showed medium to strong immunoreactivity for IGF-1R that was also demonstrated in EMA-positive perineurial cells. The intensity of staining was stronger in neurofibromas than in MPNST.

**Double-staining with anti IGF-1R and anti human CD 34.** Six tumors (4 neurofibromas, 2 MPNST) were double-stained for IGF-1R and CD34. All tissue sections included endothelial cells that showed a clear reaction with the CD34-antibody. All neurofibromas had a double staining in some areas of the sections. MPNST failed to show a double staining in this series.

**Cell culture studies (Figure 4).** In Schwann cell cultures from different patients, the expression of IGF-1R was evaluated. Four cell cultures consisted of Schwann cells only and a further 4 cell cultures were co-cultivated with fibroblasts. All cell plates showed a strong IGF-1R expression, independent of the cell culture conditions.

**Statistics.** Neurofibromas from 69 patients were evaluated. In 24 patients, exclusively dermal neuromas were diagnosed. Tumors from a further 24 patients were exclusively of the plexiform type. In 8 patients, who underwent several surgical procedures, both tumor types were diagnosed. The remaining 7 patients suffered from MPNST.

MPNST were found to express significantly lower IGF-1R than neurofibromas (r = –0.439, p < 0.002; N = 49). As expected, the proliferation index (PI) was higher in MPNST than in neurofibromas. A correlation was found in neurofibromas for the expression level of IGF-1R and the PI (r = 0.372, p = 0.021; N = 38). The comparison of the mean IGF-1R expression levels in plexiform and dermal neurofibromas revealed a higher expression for the plexiform subtype (r = 0.335; p = 0.040; N = 38).

The localization of tumors correlated with the tumor type. Plexiform neurofibromas were preferentially diagnosed as local recurrence or residual tumors. Dermal neurofibromas occurred more frequently with increasing age of patients, developed in all body regions, without restriction to certain body regions, in contrast to plexiform neurofibromas that were mainly observed in axial locations (r = –0.476; p = 0.001; N = 56). Other factors like body height or gender had no apparent effect on the variables.

**Intraindividual differences of IGF-1R expression.** In 23 patients (female: 12, male: 11) samples of 5 to 18 different tumors were available for analysis. All tumors were exclusively neurofibromas. We investigated the expression of IGF-1R related to gender, topography, and histological type (plexiform vs. dermal). The IGF-1R expression was similar in different body regions of 8 females. A considerable heterogeneity of IGF-1R expression was found in 4 of 12 females.

In 7 males, the IGF-1R expression was similar in tumors of all body regions. Again, a considerable heterogeneity of IGF-1R expression was found in 4 patients.

The comparison of mean values of staining intensity in the two groups showed that patients with a heterogeneous IGF-1R expression had a slight to moderate staining intensity (N = 8). In the second group, with less intraindividual differences in IGF-1R staining intensity, 6
Figure 1. Insulin-like growth factor 1 receptor (IGF-IR) expression in cutaneous neurofibroma of NF1. Cytoplasm of tumor cells is densely stained. IGF-IR expression in keratinocytes served as an internal control. (Magnification x100, chromogen DAB, counterstain hemalum).

Figure 2. IGF-IR expression in plexiform neurofibroma. (Magnification x100, chromogen DAB, counterstain hemalum).
Figure 3. Double staining for S-100 protein (blue) and IGF-1R (brown) expression in plexiform neurofibroma. Note cytoplasmatic staining for both antibodies in Schwann cell-derived tumors. (Magnification x100, no counterstain).

Figure 4. Expression of IGF-1R in cultured Schwann cells derived from NF1 patients. (Magnification x100, chromogen DAB (brown), counterstain hemalum).
patients showed only a slight or absence of expression, 7 showed a slight to moderate expression, and 2 patients showed a moderate to high expression. Neither gender nor histological type (plexiform, dermal) had an influence on the IGF-1R expression in this subgroup.

Discussion

In the present study it was demonstrated that several cell types of peripheral nerve sheath tumors of NF1 patients express IGF-1R. The double staining with anti-S-100 and anti-IGF-1R showed that Schwann cells in neurofibromas express the receptor with varying intensity.

Double-staining with IGF-1R and S-100-, CD34- or EMA-antibodies revealed IGF-1R expression in tumor cells and other normal cell types.

CD34-marked hematopoetic precursor cells play an important role in angiogenesis. Weiss et al. (12) performed a CD-34 expression study on healthy nerve tissue, peripheral nerve sheath tumors and neuroectodermal tumors. They showed that CD34 stained a further cell type inside the endoneurium. These cells were declared to be part of the nerve sheath, neither related to Schwann cells nor fibroblasts. Furthermore, MPNST do not contain these cells. These results were confirmed by Khalifa et al. (9) who investigated CD34 expression in neurofibromas and schwannomas of 108 patients. In routine diagnostics, CD34 antibody is used to identify endothelial cells. In this study, CD34 was expressed in endothelial cells of neurofibroma, but not of MPNST.

Proliferation assessed using the MIB-1 staining index is low in neurofibromas (<5%) and showed no differences between neurofibroma subtypes (13). The MIB-1 proliferation index was high in MPNST, exceeding 20% in all cases.

Numerous growth factors act in Schwann cell proliferation both in normal tissue and in neurofibromatosis (11, 12, 14-20, 24). Schwann cells are purported to be the key element in neurofibromas and MPNST in NF1 (20, 25-27).

Our results suggest that IGF-1R expression in these tumors is capable of transmitting mitogenic signals to the neoplastic cells since IGF-1R expression in neurofibromas correlates significantly with proliferative activity. The presence of IGF-1R does not prove that the IGF plays a role in the development of neurofibromas in NF1. However, the presence of IGF-1R in both benign and malignant nerve sheath tumors suggests that these tumors can respond to IGF. Furthermore, IGF-1R signalling is known to play a crucial role in the development and progression of cancer (28). This receptor is involved in the regulation of cell proliferation, anti-apoptosis, differentiation and cell motility (28). IGF-1R is a phylogenetically conserved receptor tyrosine kinase, ubiquitously expressed in tissues in which it plays a role in tissue growth, predominantly via the growth hormone (GH). GH receptor is expressed in neurofibromas of NF1 patients (16).

The temporal and spatial effects of these factors on tumor development in NF1 are currently under intensive investigation. Our results support the conclusion that signalling via IGF-1R should be added to the concept of tumor progression in NF1. Further studies are needed to elucidate the interactions of these factors in the development of neurofibromas and the progression of benign nerve sheath tumors to MPNST in NF1.

Acknowledgements

This study was supported in part by the Deutsche Krebshilfe.

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Received December 13, 2006
Revised March 7, 2007
Accepted March 9, 2007