Expression of the Neurofibromatosis Type 2 Gene in Human Tissues

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SUMMARY The neurofibromatosis Type 2 tumor suppressor gene is implicated in the hereditary tumor syndrome NF2, hallmarked by bilateral vestibular schwannomas, meningiomas, and ocular non-neoplastic features. The gene product has characteristics of a membrane cytoskeleton-linking protein but the mechanism of tumor suppression by the NF2 protein remains to be elucidated. The NF2 gene is widely expressed in mouse and rat tissues. In humans, most of the expression data have accumulated through Northern blot analysis, RT-PCR and, more recently, Western blot analysis, providing information on whole tissues and organs rather than on specific cell types. We report here an extensive survey of NF2 gene expression in human tissues using a combination of mRNA in situ hybridization (mRNA ISH) and immunohistochemistry (IH) with a panel of monoclonal antibodies (MAbs) supplemented by tissue immunoprecipitation experiments with affinity-purified polyclonal antibodies. Expression was observed in many different cell types, most of which appear functionally normal in individuals affected by NF2. Surprisingly, expression could not be consistently documented in Schwann cells and arachnoidal cells by IH or by mRNA ISH in formalin-fixed tissue. However, consistent immunostaining of Schwann cells was seen in frozen sections.

KEY WORDS
- neurofibromatosis type 2
- gene expression
- in situ hybridization
- immunohistochemistry
- immunoprecipitation

Cloning of the neurofibromatosis Type 2 (NF2) gene in 1993 (Rouleau et al. 1993; Trofatter et al. 1993) led to the decisive segregation of the neurofibromatosis Type 1 and neurofibromatosis type 2 syndromes. NF2 is characterized by the development of bilateral vestibular schwannomas. In addition, other central nervous system tumors, particularly meningiomas and, to a lesser extent, gliomas and ependymomas, occur in NF2 patients (Gutmann et al. 1997). Spinal tumors observed in a high percentage of cases by neuroimaging studies less commonly lead to functional impairment (Mauthner et al. 1995). Non-neoplastic features of NF2 include juvenile subcapsular lenticular opacities, retinal hamartomas, and epiretinal membranes. Mutations in the NF2 gene are detected in NF2-related and sporadic schwannomas and meningiomas, supporting its role as a classical tumor suppressor gene (Zwarthoff 1996). In addition, mutations have been detected in pleural mesotheliomas, a tumor that does not occur in NF2 patients (Bianchi et al. 1995; Sekido et al. 1995; Kleymenova et al. 1997). The 595-amino-acid NF2 protein is homologous to a subgroup of band 4.1 proteins, ezrin, radixin, and moesin, collectively known as the ERM protein family. ERM proteins function as membrane-organizing proteins, linking the plasma membrane to the cytoskeleton (Arpin et al. 1994; Vaheri et al. 1997). On the basis of homology of the NF2 protein to the ERM proteins, it has been postulated that the NF2 protein likewise functions as a membrane-cytoskeleton linking protein. The CD44 glycoprotein and a regulatory co-factor of the Na\(^+\)-H\(^+\) exchanger are candidate NF2 associating membrane proteins (Sainio et al. 1997; Murthy et al. 1998). It has been shown that the NF2 protein co-localizes with elements of the actin cytoskeleton, possibly acting, with reports supporting co-localization with F-actin in stress fibers and subcortical actin fibers (den Bakker et al. 1995b; Gonzalez-Agosti et al. 1996). Other reported putative NF2 associating cy-
toskeletal proteins include spectrin (Scoles et al. 1998), RhoA (Scherer and Gutmann 1996), and tubulin (Xu and Gutmann 1998). To date, localization studies and biochemical approaches support the postulated role of the NF2 protein, although conclusive evidence is still lacking and the method of tumor suppression is unclear.

The expression of the NF2 gene has been extensively studied in mouse and rat tissues using various techniques, including mRNA in situ hybridization (mRNA ISH), RT-PCR, immunohistochemistry, and Western blotting (Haase et al. 1994; Huynh et al. 1994; Claudio et al. 1994, 1995, 1997; Gutmann et al. 1995; Huynh and Pulst 1996). Expression studies of the NF2 gene in human tissue are limited and are mainly based on RT-PCR and Northern blotting (Rouleau et al. 1993; Trofatter et al. 1993; Arakawa et al. 1994; Bianchi et al. 1994; Hitozumatsu et al. 1994; Huynh et al. 1997; Stemmer-Rachamimov et al. 1997a, b; Takeshima et al. 1998). These approaches provide a general overview of gene expression. Detailed expression studies with visualization at the cellular level can be performed by immunohistochemistry or mRNA ISH. Immunohistochemical detection of the NF2 protein relies on the availability of suitable antibodies and the accessibility of the epitope in the tissue. Previously, we have performed immunohistochemical detection of the NF2 protein in selected human tissue samples (den Bakker et al. 1995a, b). We present here a detailed expression study of the NF2 gene in human tissue, combining mRNA ISH and immunohistochemical staining with a panel of anti-NF2 monoclonal antibodies (M Abs). This approach was adopted in favor of immunohistochemistry solely, to avoid epitope masking problems which, with respect to ERM proteins, appear to prohibit an adequate inventory (Sato et al. 1992).

Materials and Methods

Tissue Samples and Sections

For mRNA ISH and immunohistochemistry experiments, formalin-fixed, paraffin-embedded archival tissue blocks from surgical specimens were used. All brain sections were obtained from an autopsy in which there was no neurological disease. After fixation in neutral buffered 10% formalin and paraffin embedding, 5-μm sections were cut and mounted on 3-aminopropyltriethoxysilane coated slides (Sigma; St Louis, MO). Glassware and solutions were autoclaved to inhibit RNase activity; nonautoclavable material was soaked in 0.2 M HCl. All manipulations were performed under RNase-free conditions.

In Situ Hybridization Probes

Digoxigenin (DIG) labeled riboprobes BAK3AS (antisense) and BAK3S (sense) were transcribed from a subcloned 291-bp PstI fragment of the NF2 cDNA (positions 1085–1376 from the ATG initiation codon) (Figure 1). The fragment was subcloned in the pT7-19 vector (Pharmacia; Uppsala, Sweden) in two orientations. After linearization of the plasmid, transcription and digoxigenin labeling were performed according to the manufacturer’s recommendations (Boehringer Mannheim; Mannheim, Germany) using T7 RNA-polymerase. The BAK2S and BAK2AS probes (235 bp, positions 1376–1611 from the ATG initiation codon) (Figure 1) were transcribed from PCR-generated DNA templates. A consensus T7 RNA polymerase binding sequence was incorporated in the primer sets. The antisense BAK2 probe was transcribed from a DNA template generated with primers P1 (5'-AGGAAGACCGCGCGAGGCGGA-3', forward) and P2T7 (5'-TGCTTTGCTTTTCCATGGCTATCCACGATTCACATAGG-3', reverse). For the sense BAK2 probe, primers T7P1 (5'-GCATCTATACCTACTATAGGAAGACCGCGCGAGGCGGA-3', forward) and P2 (5'-TGCTTTTCCATGG-3', reverse) were used. The PCR products were purified (Qiaquick spin column; Qiagen, Hilden, Germany) and used for transcription. Transcribed riboprobes were checked by agarose gel electrophoresis, ethidium bromide staining, and spot-blotting. Initial hybridization experiments performed on parallel tissue sections with BAK3AS/S and BAK2AS/S indicated that the results with these two probe sets were comparable, with the BAK3AS/S probe set generally generating a stronger signal. All further hybridizations were carried out with probe set BAK3AS/S.

In Situ Hybridization

mRNA in situ hybridization was performed essentially as described by Lindenbergh et al. (1997). Hybridization was performed at 53°C (2 × SSC, 50% formamide) in a humid chamber overnight with a final probe concentration of 100 ng/ml. Posthybridization washes were carried out at 45°C. Visualization of RNA hybrids was achieved by incubation with alkaline phosphatase-conjugated anti-digoxigenin antibodies (Fab fragments; Boehringer Mannheim); 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-chloro-indolyl-phosphate (BCIP) were used as substrate. Color development was performed in the dark with periodic visual inspection. The reaction was terminated when a sufficiently strong signal was observed. The sections were counterstained with nuclear fast red, dehydrated through ethanol gradients and mounted with Euparal (Chroma-Gesellschaft; Stuttgart, Germany). Control hybridizations were performed in selected cases with the DIG-labeled insulin-like growth factor-binding protein 5 (IGF-BP5) probe (Schuller et al. 1993), kindly provided by D. Lindenbergh-Kortleve. Control slides were

![Figure 1: Schematic diagram of the NF2 protein with relative positions of synthetic peptides and mRNA ISH probe sets.](https://example.com/figure1.png)
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Antibodies and Immunohistochemistry
The production of the anti-NF2 MAb KF10 has been described previously (den Bakker et al. 1995b). Production of M Abs FE9, HB7, and AH10, immunohistochemistry, and ELISA were performed as described previously (den Bakker et al. 1995b). In ELISA and immunohistochemical control experiments, culture supernatant from the IgG1-secreting myeloma parent cell line (P3-X63Ag8) was used. M Ab FE9 was raised against synthetic peptide SPN F2-0 (den Bakker et al. 1995b) and M Abs HB7 and AH10 were raised against synthetic peptides SP276 and SP277, respectively (Figure 1) (den Bakker et al. 1995a). The isotype of the M Abs was determined with the use of the Isostrip isotyping kit (Boehringer Mannheim). Primary M Abs AH10 and HB7 were used as undiluted culture supernatants. FE9 and KF10 were used at 1:50 to 1:100 dilutions from culture supernatants from a Tecnomouse incubator (Tecnomara; Integra Biosciences, Wallisellen, Switzerland); all antibody incubations were performed overnight at 4C. For immunoperoxidase staining, the peroxidase-anti-peroxidase (PAP) method was used. Staining intensity for FE9 was improved by wet heat (microwave antigen retrieval) pretreatment. As a chromogen, diaminobenzidine (DAB) was used. Sections were incubated in the chromogen solution with hydrogen peroxide as a substrate for no longer than 7 min in the dark with gentle agitation. After rinsing, sections were counterstained with hematoxylin. No enhancement techniques were used. Negative controls included omission of the primary antibody, staining of parallel sections with undiluted culture supernatant from the myeloma parent cell line which secretes an IgG1 antibody, and staining with a commercially available antibody. The sections stained with culture supernatant from the myeloma parent cell line was seen with the MAb AH10 and, to a lesser extent, acellular stroma was stained with this antibody. The sections stained with culture supernatant from the myeloma parent cell line was seen with the MAb AH10 and, to a lesser extent, acellular stroma was stained with this antibody. Consistent NF2 gene expression was observed in a number of tissues by mRNA ISH, immunohistochem-

mRNA In Situ Hybridization and Antibody Controls
The specificity of the probes was checked by control hybridizations on NF2- and mock-transfected COS cells with and without prior DNase or RNase digestion. A strong cytoplasmic hybridization signal was observed in approximately 10% of the cells (not shown). No signal was observed after RNase digestion, but DNase digestion did not alter staining, indicating that the signal was not due to hybridization of the riboprobe to transfected NF2 cDNA. Weak background staining in all COS cells was observed with the antisense and sense probes in most experiments.

Antibodies FE9 and AH10 were determined to be IgG1κ antibodies, while HB7 was an IgMκ antibody. In solid-phase assays (ELISA) using synthetic NF2 peptides and nonrelevant protein (bovine serum albumin), the M Abs reacted exclusively to the NF2 peptides to which these had been raised. No reactivity in ELISA was seen when culture supernatant from the myeloma parent cell line was used. All M Abs and affinity-purified polyclonal antibodies detected the 69-kD band in Western blots of NF2-transfected COS cells. In Western blotting experiments and immunocytochemical staining experiments using cultured epithelial cell lines, we found that the anti-SP-0 antibody FE9 crossreacts with a low molecular weight cytokeratin (not shown). Therefore, staining of epithelia with this antibody was not interpreted. Staining of nuclei in some tissue sections was seen with the MAb AH10 and, to a lesser extent, acellular stroma was stained with this antibody. The sections stained with culture supernatant from the myeloma parent cell line demonstrated weak nonspecific staining that did not overlap with staining patterns achieved by the NF2 M Abs. No signal was observed in sections stained with anti-PSA antibodies.

NF2 Expression in Tissues by mRNA ISH and Immunohistochemistry
Consistent NF2 gene expression was observed in a number of tissues by mRNA ISH, immunohistochem-
isty, and immunoprecipitation. An overlapping but distinct staining pattern of the various anti-NF2 antibodies was found.

Epithelium and Mesothelium. High levels of NF2 gene expression were observed in all layers of the epidermis, excluding the acellular cornified layer, by ISH (Figure 2A). Noncornifying stratified epithelium of the esophagus also stained. However, here the ISH signal was limited to the lower layers (Figure 2B). A strong ISH signal was observed in urothelium and in the epithelium of the small intestine. In the small intestine, the signal was confined to the crypts and diminished towards the tips of the villi (Figure 2D). Weak ISH staining was observed in colon epithelium, generally located at the basal parts of the crypts. Other epithelia in which a weaker mRNA ISH signal was observed included pseudostratified epithelium of the trachea, bronchial epithelium, epithelium of eccrine sweat ducts in the skin, acinar cells in the pancreas, cells of the collecting ducts in the kidney, and follicular epithelium in the thyroid and parathyroid glands. No ISH signal was observed in fat, cardiac muscle, cartilage, liver, prostate, adrenal gland, spleen, thymus, and peripheral nerve. Immunostaining closely mimicked mRNA ISH staining in stratified epithelia, with strong staining by AH10, HB7, and KF10 (Figure 2C). In nonstratified epithelia, weak immunostaining was observed with AH10 only.

Hybridization and immunostaining of peritoneal mesothelial cells were observed, but of variable intensity (Figure 2E). Mesothelial cells in other locations, including pleura and epicardium, were also stained with NF2 antibodies. A stronger cytoplasmic immunohistochemical signal was observed when cytological smears from pleural aspirates were used (immunohistochemistry only; Figure 2F).

Neural Tissue. Strong and consistent immunohistochemical and mRNA ISH staining was observed of neurons in the autonomic ganglia throughout the intestinal tract (Figure 2G). However, Schwann cells and satellite cells were negative. Likewise, Schwann cells in other locations, e.g., in peripheral nerves or in peripheral sections of cranial nerves, did not show any hybridization signal (Figure 2H), and only weak and nonconsistent immunostaining was seen in paraffin sections. However, immunostaining of cryostat sections of peripheral nerve with FE9 was consistently positive (Figure 2I). In the central nervous system, neurons were consistently positive with mRNA ISH but no regional differences were noted. Cortical neurons, neurons of cranial nerve ganglia, and neurons of extrapyramidal locomotory systems stained with equal intensity (Figure 2J). We did observe diminishing staining intensity towards the deeper layers, most likely due to impaired penetration of fixative into the brains after autopsy. Weak and inconsistent ISH staining of glial cells was noted. A weak hybridization signal was observed in ependymal cells, but arachnoidal cells were negative. Staining of neurons with the NF2 MAb was not observed, and no immunostaining of arachnoidal cells was seen. In the cerebellum, the Purkinje cells hybridized strongly (Figure 2K) but no ISH staining of the molecular or granular layer was observed. Immunostaining of cells in the cerebellum was not seen. In the human eye, a hybridization signal was observed in amacrine cells in the retina. Very weak ISH staining was observed in the cornea and in lens epithelium, whereas immunostaining was negative.

Blood Vessels. Variable NF2 gene expression was found in endothelium in blood vessels. In blood vessels located in the loose subserosal tissue in the gastrointestinal tract and in umbilical cord vessels, strong specific endothelial ISH and immunostaining with the FE9 MAb was observed, but not with any of the other antibodies (Figure 2L–2N). In other tissues, staining of endothelial cells was weak or absent. Of note was the very weak or complete absence of an ISH signal in the smooth muscle cells of the tunica media of blood vessels, despite consistent strong positive immunostaining with several antibodies (Figure 2L–2N). Skeletal and cardiac muscle demonstrated low expression levels of immunostaining with one antibody (AH10), but ISH was completely negative.

Other Tissues. No staining was seen in liver, including bile duct epithelium, spleen, prostate epithelium, thymus (only staining of Hassal’s corpuscles), adrenal gland, pneumocytes, circulating blood cells, fat, cartilage, mucous glands in esophagus and trachea, islet cells and ductal epithelium in the pancreas, or in glomeruli and renal tubules. Immunohistochemical staining was seen of tissue macrophages in various tissues. In lymph nodes, immunostaining of high endothelial venules was seen.

Immunoprecipitation

To confirm the presence of NF2 protein in various tissues, we performed immunoprecipitation experiments with affinity-purified anti-NF2 antibodies. Frozen tissue samples were available for immunoprecipitation experiments for a selection of tissues. In all tissue samples, NF2 could be immunoprecipitated by either of the affinity-purified polyclonal antibodies A19 and 1399A (Figure 3).

Immunoprecipitated protein could be detected with any one of the MAbs and with either of the affinity-purified polyclonal antibodies. In addition to the expected 69-kD band, a slower migrating band of approximately 130 kD was observed in most samples,
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Figure 2 Immunohistochemical and mRNA ISH staining of human tissue. (A) mRNA ISH of epidermis, BAK3-AS probe. Original magnification ×200. (B) mRNA ISH of esophagus, BAK3-AS probe. Original magnification ×100. (C) Immunostaining of epidermis, KF10 antibody. Original magnification ×400. (D) mRNA ISH of small intestine, BAK3-AS probe. Original magnification ×100. (E) mRNA ISH of mesothelial lining of intestine, BAK3-AS probe. Original magnification ×200. (F) Immunostaining of pleural aspirate, FE9 antibody. Note strong staining of mesothelial cells and weaker staining of histiocytes; lymphocytes do not stain. Original magnification ×400. (G) mRNA ISH of intestinal ganglion, BAK3-AS probe. A clear signal is seen in neurons; satellite cells do not stain. Original magnification ×200. (H) mRNA ISH of peripheral nerve, BAK3-AS probe. ISH staining is seen in endothelial cells; Schwann cells are negative. Original magnification ×200. (I) Immunostaining of cryostat section of peripheral nerve in subepicardial connective tissue, FE9 antibody. Note strong cytoplasmic staining of Schwann cells and weaker staining of mesothelial (epicardial) lining. Original magnification ×200. (J) mRNA ISH of CNS neurons, BAK3-AS probe. Very weak staining of glial cells is observed in addition to strong cytoplasmic staining of neurons. Original magnification ×400. (K) mRNA ISH of Purkinje cells in the cerebellum, BAK3-AS probe. Original magnification ×400. (L) Immunostaining of blood vessel, KF10 antibody. The tunica media is stained; the endothelial lining does not stain with this antibody. Original magnification ×200. (M) mRNA ISH of blood vessels in loose subserosal tissue in the intestinal tract, BAK3-AS probe. Note absence of staining of smooth muscle cells of the tunica media; a strong hybridization signal is seen in endothelial cells. Original magnification ×200. (N) Immunostaining of blood vessel, FE9 antibody. Both smooth muscle cells in the tunica media and endothelial cells are stained. Original magnification ×200.
and in lysates of esophagus and adrenal gland a band of 250 kD was also seen. These additional bands were detected by several NF2 antibodies and therefore may represent NF2 dimers. In most lysates, the 69-kD NF2 band presented as a doublet, probably due to phosphorylation. Two human smooth muscle cell lines, one an established smooth muscle cell line derived from the muscularis externa of the intestine [human intestinal smooth muscle, HISM (Graham et al. 1984)] and a second primary smooth muscle culture from the human airway (kindly supplied by M s. S. M Kay; Department of Pharmacology, Erasmus University, Rotterdam), were used for immunoprecipitation. Both cell lines yielded a 69-kD band that was detected with MAb s raised against distinct NF2 peptide epitopes (not shown). In some experiments, the 69-kD band presented as a doublet.

Discussion

Neurofibromatosis Type 2 is characterized by the development of schwannomas of the VIII cranial nerves and by meningiomas. Schwannomas are benign tumors arising from neural crest-derived Schwann cells, in which inactivation of the NF2 gene is believed to be paramount in pathogenesis. Therefore, expression of the NF2 gene is expected in Schwann and arachnoidal cells.

To further document NF2 gene expression in human tissue, we performed immunohistochemical and mRNA ISH staining experiments on an extensive panel of human tissues. Although frozen sections may provide increased sensitivity, we used formalin-fixed, paraffin-embedded sections for all experiments to ensure a comprehensive tissue panel and optimal morphology after hybridization. An exception was made to demonstrate the presence of NF2 protein in Schwann cells in peripheral nerve. In this case, both formalin-fixed and cryostat sections were used for immunohistochemical staining. The discrepancy of nonstaining tissue in paraffin sections vs clear immunoreactivity in cryostat sections is well known. In a number of tissues in the study presented here, including liver, spleen, adrenal gland, thymus, muscle, prostate epithelium, cartilage, pneumocytes, and circulating blood cells, we could not document NF2 expression by either mRNA ISH or IH. It is possible that the sensitivity of the methods employed is insufficient to detect the presence of NF2 protein in these tissues. Therefore, we interpreted positive immuno- or ISH staining as evidence of NF2 gene expression, although acknowledging that lack of staining does not rule out NF2 gene expression below the detection threshold levels.

Expression studies of the NF2 gene have been performed by a number of techniques including Northern blotting, RT-PCR, Western blotting, mRNA-ISH and immunohistochemistry. Furthermore, the majority of these investigations have been performed on non-human tissues, in particular rodent tissues and on cell lines (Rouleau et al. 1993; Trofatter et al. 1993; Arakawa et al. 1994; Bianchi et al. 1994; Claudio et al. 1994,1995,1997; Hase et al. 1994; Hidotsumatsu et al. 1994; Huynh et al. 1994,1996,1997; Sainz et al. 1994; Gutmann et al. 1995; Stemmer–Rachamimov et al. 1997a,b; Takeshima et al. 1998). A drawback of a number of techniques utilizing tissue homogenates, such as Northern blotting, Western blotting, and RT-PCR, is the inherent lack of detail. No information on specific cell types can be inferred from these experiments. Furthermore, until evidence exists that the NF2 protein functions similarly in rodent cells and human cells, comparisons of expression studies should be interpreted with care.

Strong and consistent immuno- and ISH staining of epithelia, particularly stratified epithelia, was observed. Expression was observed both in keratinizing and nonkeratinizing stratified epithelium. Previously, we have documented co-localization of NF2 protein with keratohyalin granules and intermediate filaments in keratinocytes (den Bakker et al. 1995b). Apart from cutaneous schwannomas, NF2 patients do not develop specific skin disorders. Our findings coincide with immunohistochemical and mRNA ISH data from a developmental study in mice in which expression in keratinocytes in the epidermis was described (Huynh et al. 1996).

NF2 gene mutations have been documented in malignant mesothelioma (Bianchi et al. 1995; Sekido et al. 1995; Kleymenova et al. 1997), a tumor that is not seen with increased frequency in NF2 patients. In support of the involvement of the NF2 gene in the development of malignant mesothelioma is our finding of...
NF2 gene expression in pleural, epicardial, and abdominal mesothelium. It remains to be elucidated if, comparable to epithelium, the NF2 protein co-localizes and associates with intermediate filaments in mesothelial cells.

Schwann cells are derived from the neural crest and form myelin sheaths around axons in the peripheral nervous system. Sporadic and NF2-related schwannomas arise from Schwann cells. Therefore, the presence of NF2 protein is expected in Schwann cells. Surprisingly, no signal was observed in Schwann cells with either of the hybridization probe sets, and immunostaining was weak and variable in paraffin sections. However, strong and consistent positive immunostaining of Schwann cells was seen in frozen sections. Approximately half of NF2 patients develop meningiomas. These tumors, with variable histological appearance, are generally believed to originate from arachnoidal cells in the leptomeninges. Therefore, it is assumed that the NF2 gene is expressed in these cells. We were not able to demonstrate NF2 gene expression in arachnoidal cells with mRNA ISH, nor did we detect NF2 protein by immunohistochemical methods with the M Abs. The lack of reactivity of Schwann and arachnoidal cells in mRNA ISH and immunostaining experiments may be caused by insufficient sensitivity of the employed methods to detect low levels of NF2 mRNA and protein. In previous reports the presence of the NF2 protein in Schwann and arachnoidal cells has been demonstrated by immunohistochemical methods (Sainz et al. 1994; Claudio et al. 1995; Scherer and Gutmann 1996; Huynh et al. 1997; Stemmer–Rachamimov et al. 1997a,b). However, the results of these experiments are not consistent, and staining in some reports has been achieved only by very sensitive methods. Investigators using more conventional staining techniques describe cytoplasmic perinuclear staining in some Schwann cells and variable expression in arachnoidal cells in frozen sections of human tissue (Stemmer–Rachamimov et al. 1997a). In addition, fixation time appears critical for immunohistochemical detection of NF2 protein (Scherer and Gutmann 1996), and it appears that detection of NF2 protein in tissues is dependent on the epitope to which antibodies are reactive. Several investigators have used cultured Schwann cells to detect NF2 protein. It must be borne in mind, however, that ERM gene expression may be upregulated in cell culture, while corresponding endogenous tissue expression has not been documented for the particular ERM protein (Amieva and Furthmayr 1995; Hirao et al. 1996; Tsukita et al. 1997). Previously, it has been demonstrated that NF2 gene expression in the mouse and possibly in humans is developmentally regulated (Rouleau et al. 1993; Huynh et al. 1996). Failure of demonstration of NF2 expression in the study in adult human tissue could therefore be due to physiological downregulation in Schwann and arachnoidal cells.

As we and others have noted, the NF2 gene is expressed in smooth muscle cells and endothelial cells (Claudio et al. 1995; den Bakker et al. 1995a; Stemmer–Rachamimov et al. 1997a). Therefore, expression studies utilizing RT-PCR, Northern blotting and, to a certain extent, Western blotting may erroneously detect NF2 expression originating from blood vessel wall elements in tissues not truly expressing the NF2 gene.

Comparable to findings in rodents, we found a strong ISH signal for the NF2 gene in neurons of the central nervous system, including the Purkinje cells in the cerebellum. In contrast, we were not able to detect NF2 protein in these cells using antibodies to various epitopes. It is possible that the antibodies we employed were directed to masked epitopes. Indeed, a recent report describes extensive expression of the NF2 gene in the human CNS by immunohistochemical methods using an MAb directed to C-terminal NF2 sequences (Stemmer–Rachamimov et al. 1997b). In addition to neurons, these authors detected NF2 protein in glial and ependymal cells. Although we did occasionally observe an ISH signal in glial cells, this was not readily reproducible, and weak ISH staining was also observed after hybridization with sense probes.

It appears that the NF2 gene is expressed in many human tissues, most of which are not involved by disease in individuals affected by NF2. NF2 gene mutations, in particular, affect Schwann cells and arachnoidal cells and do not affect other tissues that apparently express the NF2 gene. It therefore appears plausible that NF2 expression in these tissues may be redundant. Experiments providing support for this hypothesis have been performed by Takeuchi, Huynh, and Henry (Takeuchi et al. 1994; Henry et al. 1995; Huynh and Pulst 1996). By introducing antisense NF2 oligonucleotides into the Schwann cell-like cell line ST-S-26, Huynh et al. observed morphological and stimulatory proliferation effects, and Takeuchi et al. showed that inhibition of all three ERM proteins was required in thymoma and epithelial cells for maximal inhibition of cell adhesion and for morphological changes. Why this putative redundancy might fail in Schwann cells and arachnoidal cells is not clear. Although cell type-specific expression of a single ERM protein seems an attractive explanation, Schwann cells have been shown to express moesin (Stemmer–Rachamimov et al. 1997a) as well as NF2 protein, thus excluding this hypothesis. An alternative explanation for the apparent discrepancy between NF2 disease symptoms and tissue expression patterns of the NF2 gene may be that, in analogy to loss of the retinoblastoma gene in several epithelia, loss of function of NF2 function in epithelial cells induces hyperproliferation masked...
by an increased rate of apoptosis (Williams et al. 1994).

We have provided an inventory of human NF2 gene expression. From this inventory and the available literature data, it can be concluded that NF2 and ERM genes are indeed expressed in a partially overlapping set of tissues. Additional studies are required to focus on common and distinct NF2 and ERM protein functions.

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