3.3.3 Stable cell lines expressing *Ptpns1* or *Cd47*

To study the potential role of PTPNS1 and CD47 in migration and differentiation of neural progenitors, NIH/3T3 fibroblasts were stably transfected to constitutively express either *Ptpns1* or *Cd47*. These cell lines produced fusion proteins with two tags at the C-terminus: a V5-tag and a His-tag. These tags facilitated the detection of the proteins in immunofluorescence and immunoblotting and allowed to distinguish the endogenous from the overexpressed proteins. To generate stable cell lines, transfected cells that expressed the fusion proteins were selected with the antibiotic Zeocin. Resistant clones were propagated and tested for the production of the two proteins by immunofluorescence and immunoblotting. Whereas many clones expressing *Ptpns1*-V5-His were recovered, only a few stably expressed *Cd47*-V5-His. The molecular masses deduced from the immunoblot analyses fitted published data, demonstrating proper glycosylation of the fusion proteins (Fig. 20 a and c).

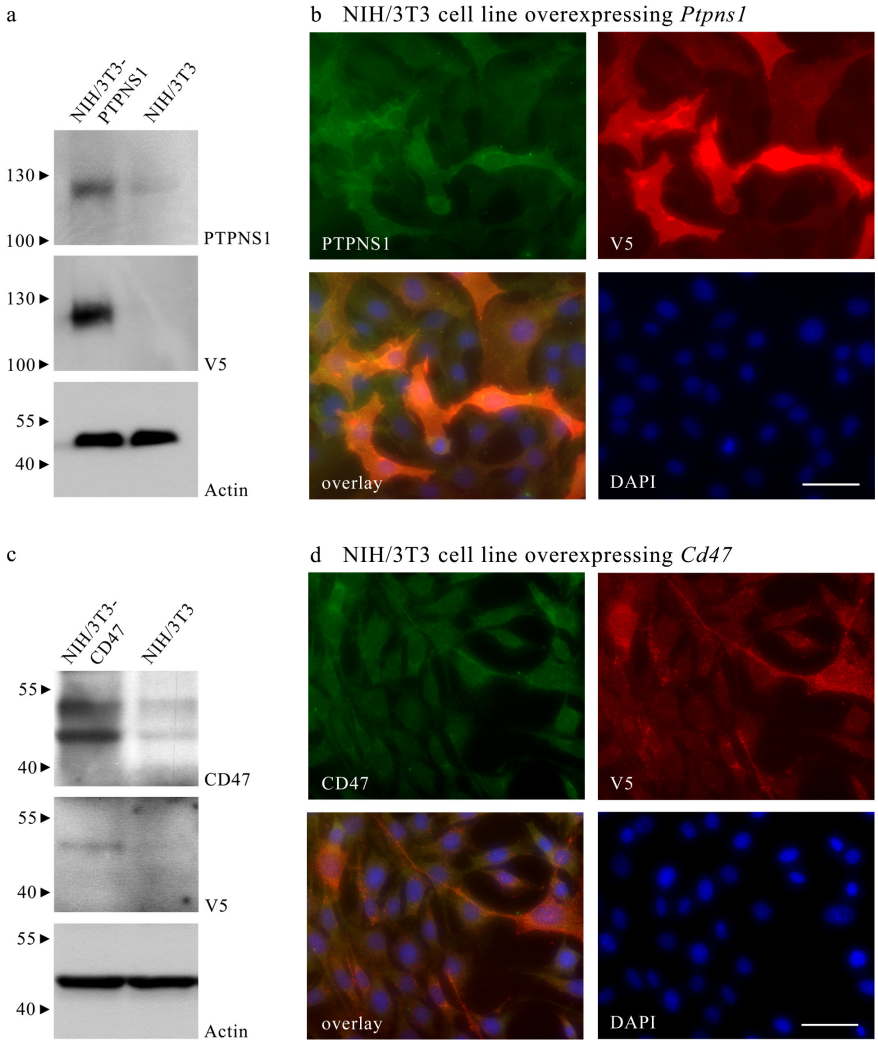

Figure 20. NIH/3T3 fibroblasts stably expressing *Ptpns1* or *Cd47*. Cell lysates from NIH/3T3 cells with and without transgenes were subjected to immunoblot analysis with antibodies against PTPNS1, CD47, V5, and actin (a, c). Both PTPNS1 and CD47 were weakly produced endogenously in fibroblasts without transgenes, and enhanced expression was detected in the clonal cell lines. Detection of the V5-tag permitted discrimination between overexpressed and endogenous protein. Actin synthesis was visualized to demonstrate equal amounts of protein in each lane. The molecular weight in kDa as deduced from the weight marker is indicated on the left. Immunofluorescence analysis showed synthesis of PTPNS1 and CD47 in the engineered cell lines (b, d). Staining of PTPNS1 or CD47 (green), V5-tag (red) and DNA (blue), and an overlay of all three colors are depicted. Scale bar equals 50 µm.

Immunofluorescence studies showed that the signals from the protein-specific antibodies and the V5-tag antibody overlapped (Fig. 20 b, d). Although all cells of a clonal line produced the respective fusion protein, total amounts of these seemed to vary within the culture. High over-expression of either *Ptpns1* or *Cd47* decreased the viability of the fibroblasts and their proliferation rate, as was revealed by transient transfection experiments (data not shown).

Interestingly, two protein bands were detected with the CD47-specific antibody by immunoblotting (Fig. 20 c). Both signals were enhanced in the clonal cell line, indicating that both are specific and correspond to CD47. Since the antibody specific for the C-terminal V5-tag only detected the larger protein, and the CD47-specific antibody binds to the N-terminus of the protein, the smaller protein visualized in the blot could correspond to an N-terminal fragment of CD47. This indicates that CD47 might be processed post-translationally to yield two fragments.

The interaction of PTPNS1 and CD47 is known to play a role in cell migration. Purified CD47 inhibited migration of human melanoma cells and of CHO cells *in vitro*, and over-expression of *Ptpns1* in these cells supported their migration (Motegi et al., 2003). From own previous pilot experiments it was established that neurosphere cells attach to fibroblast monolayers and migrate as well as differentiate readily on top of these cells (data not shown). To test whether an excess of external PTPNS1 or CD47 presented to the neural progenitors alters their migratory behavior *in vitro*, clonal fibroblast cell lines and untransfected fibroblasts were cultivated until they reached confluency and neurospheres were then placed on top of this monolayer (Fig. 21). Migration and differentiation were visualized by immunofluorescence analysis using anti-tubulin-β-III and anti-GFAP antibodies. Fibroblasts do not express these proteins at detectable amounts, as was tested in previous experiments, thereby allowing to distinguish them from neural cells.

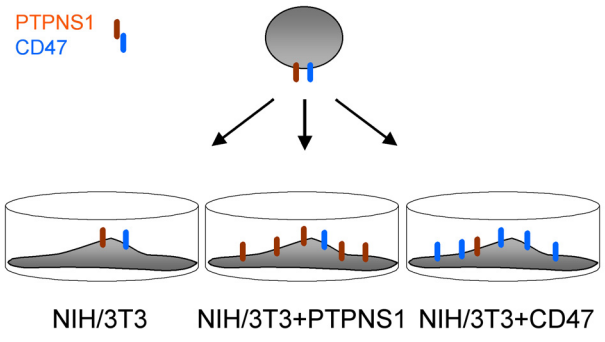

Figure 21. Neural progenitor cell migration and differentiation on fibroblast monolayers. Neural progenitors in neural progenitor medium lacking EGF were placed on top of confluent monolayers of NIH/3T3 fibroblasts. Control fibroblasts endogenously expressed *Ptpns1* and *Cd47* at low levels. In the clonal cell lines, expression of either *Ptpns1* or *Cd47* was strongly enhanced.

The neurosphere cells attached to the monolayers and migrated away from the spheres. Some of the cells acquired a neuronal morphology with long cellular processes and synthesized tubulin-β-III (Fig. 22). Other cells derived from the neurospheres were positive for GFAP (Fig. 22). When

the differentiation of neural progenitors on untransfected fibroblasts was compared to that on clonal fibroblasts producing PTPNS1 or CD47, no difference in migration was observed. Also the proportion of cells expressing tubulin-β-III was similar. Therefore, an excess of PTPNS1 or CD47 did not seem to influence the behavior of neural progenitor cells *in vitro* when migrating and differentiating on fibroblast monolayers.

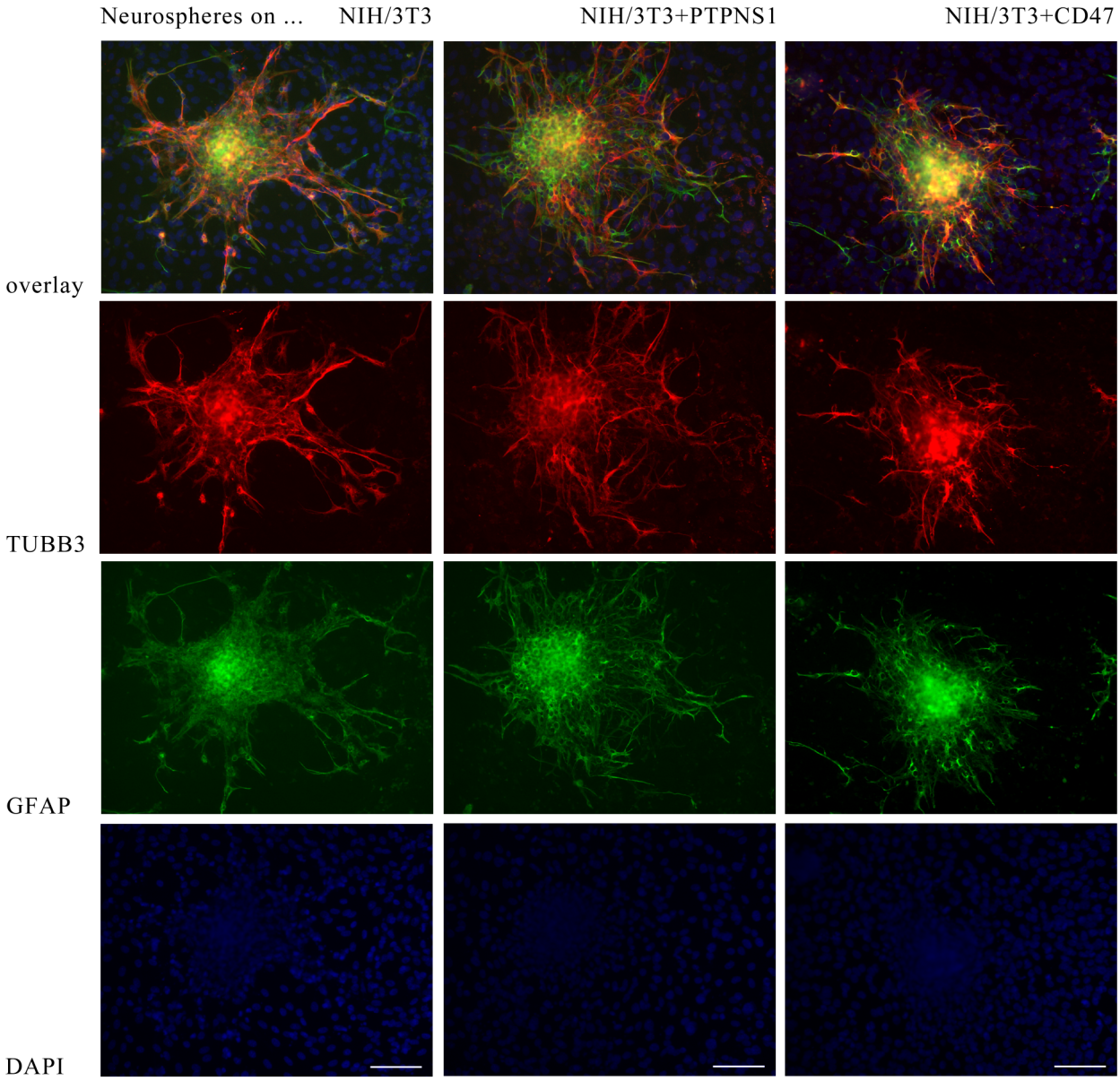

Figure 22. Immunofluorescence analysis of neurosphere cell migration and differentiation on fibroblast monolayers producing an excess of PTPNS1 or CD47. NIH/3T3 fibroblasts and clonal cells derived from these were grown to confluency. Neurospheres were plated onto the monolayers. They attached to the fibroblasts, migrated away from the spheres, and differentiated. After four days, tubulin-β-III (TUBB3, red) and GFAP (green) were stained by immunofluorescence. DAPI labeled the nuclei of fibroblasts and progenitors (blue). Whereas fibroblasts were negative for tubulin-β-III and GFAP, differentiated progenitor cells expressed one of them, demonstrating them as being neuronal or glial cells. Scale bars equal 50 µm.

Recently, Ohnishi et al. presented evidence that the transmembrane protein PTPNS1 undergoes ectodomain shedding *in vitro* (2004). They demonstrated that the ectodomain presented on the cell surface was cleaved off in CHO-Ras cells stably expressing *Ptpns1*. This disrupted the interaction of PTPNS1 with CD47 and supported migration of the cells. The ectodomain was purified from the supernatant and the site of clipping was demonstrated to be just above the membrane-spanning fragment of the protein. Thus, it was proposed, that ectodomain shedding could provide a rapid mechanism to induce migration.

Similarly, supplying additional PTPNS1 ectodomains could have an influence on the migration or differentiation of neural progenitors. Such an effect could be achieved by saturation of free CD47 receptors with additional PTPNS1 binding domains in the medium. It could also be mediated by breaking the association of PTPNS1 and CD47 due to an excess of PTPNS1 binding domains, which compete for the CD47 receptors with the PTPNS1 on the progenitor cells. Since cells clip off the PTPNS1 ectodomain *in vitro*, conditioned media of *Ptpns1* overexpressing cells should contain free ectodomains, as was shown by Ohnishi et al. (2004). Thus, PTPNS1 ectodomains should be present in the medium of the clonal NIH/3T3 cell line expressing *Ptpns1*. These could alter neural progenitor migration or differentiation. To test for such an effect, clonal fibroblasts overexpressing *Ptpns1* or *Cd47* as well as control fibroblasts were kept for two days to allow expression of the fusion proteins and possibly secretion of ectodomains into the medium. These conditoned media were removed. Neurospheres were resuspended in these conditioned media and plated into poly-L-lysine-coated dishes for differentiation (Fig. 23). To visualize any differences in migration or differentiation, cells were immunostained for tubulin-β-III and GFAP. The migration and differentiation seemed to be unaltered when comparing cells in unconditioned medium to those in medium conditioned by untransfected fibroblasts or in conditioned media from clonal cell lines (Fig. 24).

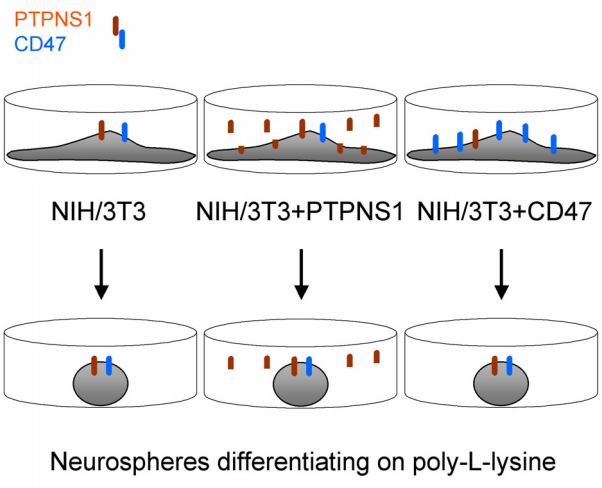

Figure 23. Neural progenitor cell differentiation and migration in conditioned media of *Ptpns1* or *Cd47* overexpressing NIH/3T3 fibroblasts. Control fibroblasts and clonal cell lines with enhanced expression of either *Ptpns1* or *Cd47* were cultivated for two days to allow production and potential shedding of PTPNS1. Supernatants of these fibroblast cultures were sterile-filtered. Neurospheres were resuspended in the supernatants and placed in dishes coated with poly-L-lysine.

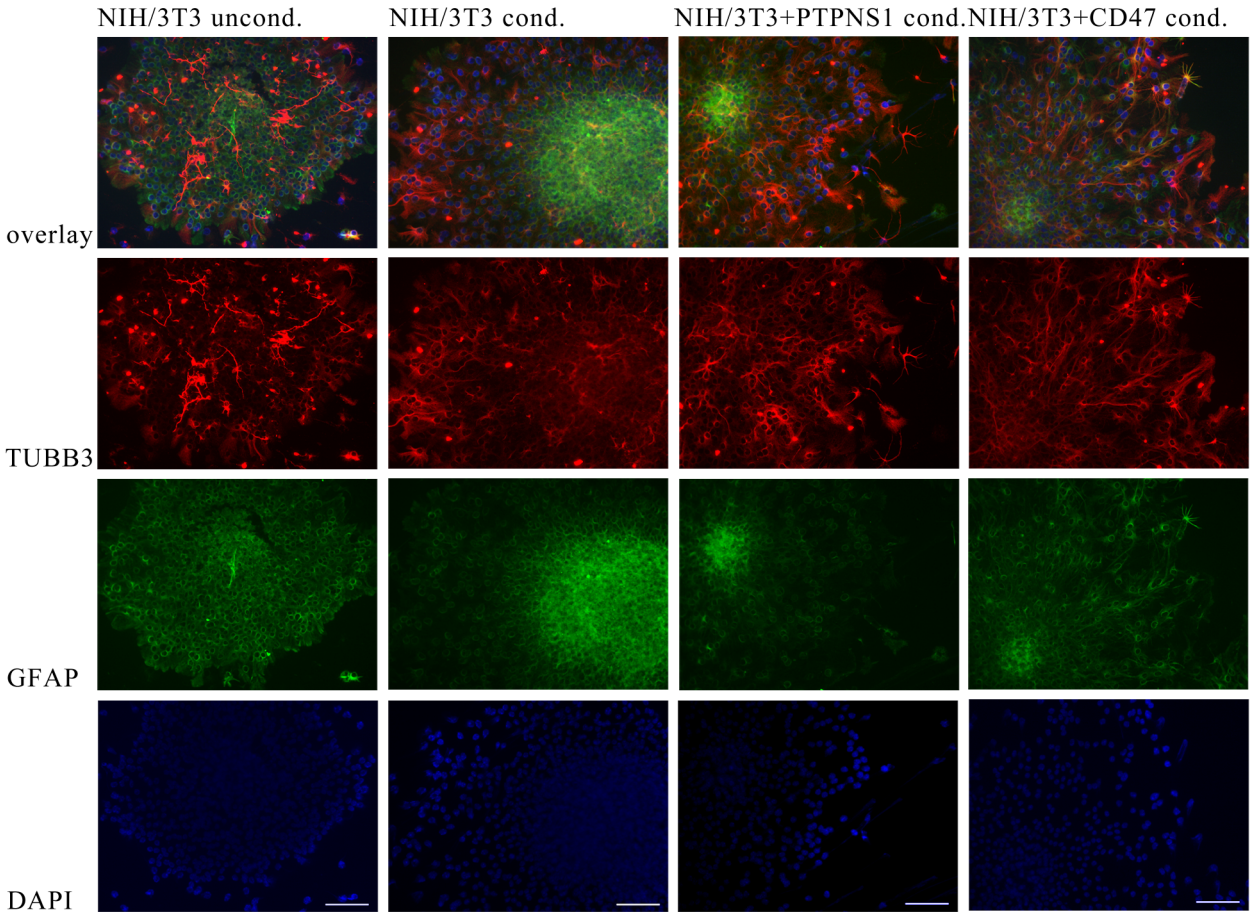

Figure 24. Neurosphere cell migration and differentiation after six days in conditioned media from NIH/3T3 fibroblasts expressing *Ptpns1* or *Cd47*. Neurosphere cells were plated in unconditioned medium and in conditioned media from control fibroblasts or clonal cell lines producing an excess of PTPNS1 or CD47. Neurosphere cells attached to the surface coated with poly-L-lysine, migrated away from the cluster, and differentiated. Cells were stained with antibodies against tubulin-β-III (TUBB3, red) and GFAP (green) by immunofluorescence. Cells close to the cluster were mainly GFAP-positive, whereas migrating cells were mostly tubulin-β-III-positive. DAPI stained the nuclei (blue). Scale bars equal 50 µm.

Interestingly, after differentiation for six days, more cells were present in conditioned media compared to those in the unconditioned medium. This was unexpected, since neural progenitors stopped proliferation under these differentiation conditions in all previous experiments. In addition, it appeared that the conditioned media from the clonal cell lines had a stronger proliferative effect compared to the control fibroblasts. To verify these observations, differentiated neurosphere cells were stained for Ki-67, a nuclear protein that labels dividing cells (Fig. 25 a). Almost no cells were dividing in unconditioned medium. In contrast, several Ki-67-positive cells were found in the cultures kept in conditioned media. The differences between the numbers of dividing cells were not only significant between unconditioned and conditioned media, but also between conditioned media from control and clonal fibroblasts overexpressing *Ptpns1* or *Cd47* (Fig. 25 b).

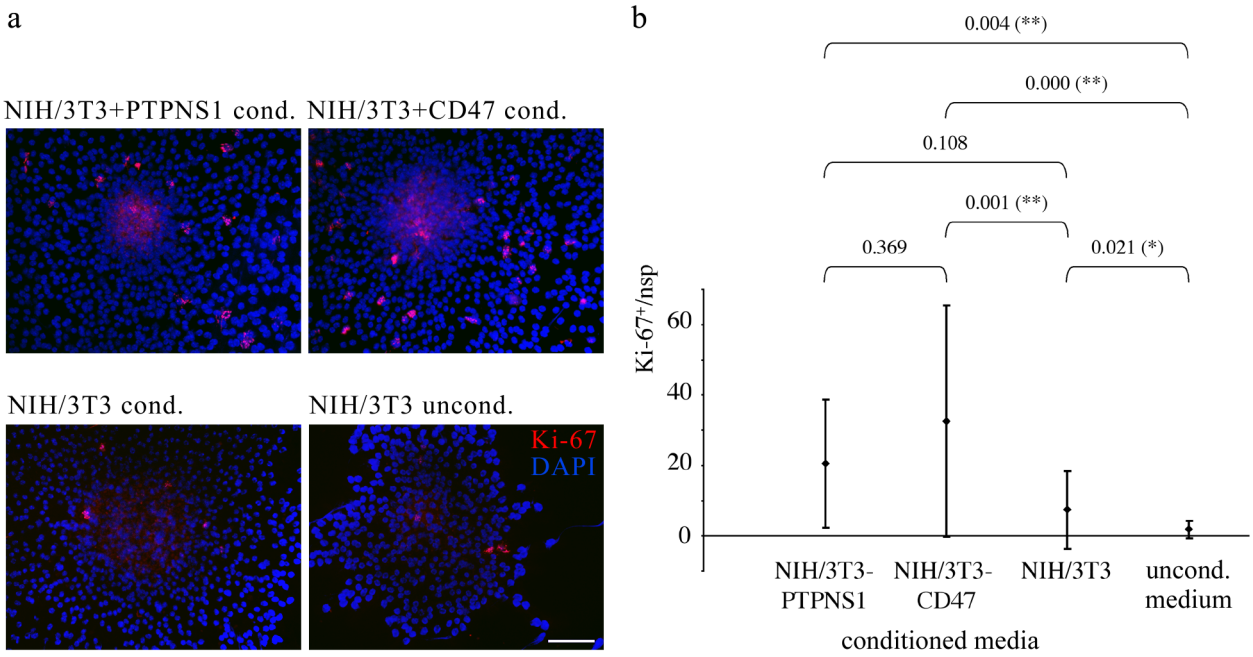

Figure 25. Detection of proliferating (Ki-67-positive) neurosphere cells in conditioned media from NIH/3T3 fibroblasts expressing *Ptpns1* or *Cd47*. (a) Neurosphere cells were kept in unconditioned medium and in media conditioned by control fibroblasts or clonal cell lines producing an excess of PTPNS1 or CD47. Neurosphere cells attached to the surface, migrated away from the cluster, and differentiated. Ki-67 synthesis was visualized by immunofluorescence after six days (red). Whereas almost no cells from the unconditioned medium presented Ki-67, some cells were positive for the proliferation marker when kept in conditioned media. Nuclei were labeled with DAPI (blue). Scale bar equals 50 µm. (b) Cells positive for Ki-67 were counted in twenty colonies derived from a single neurosphere. Arithmetic mean and standard deviation from the counted Ki-67$^+$ cells per neurosphere were calculated (y-axis). Significance of the observed differences in proliferation was analyzed using the Mann-Whitney Test (U-Test). *P*-values are given for each combination to determine the significance of the observed differences. (*) means $P<0.05$; (**) means $P<0.01$.

Does this argue for an effect of potentially shedded ectodomains? The proliferative effect was seen in all conditioned media. This would indicate, that the murine NIH/3T3 fibroblasts secrete factors, which stimulate the proliferation of neurosphere cells *in vitro*. The proliferative effect was stronger in conditioned media from the clonal cell lines compared to the conditioned medium by the control fibroblasts. These clonal cell lines were derived from single isolated cells, which stably expressed the transgene after transfection and showed a high proliferation rate. Therefore, clones might have been selected that are particularly strong in autoinduction of proliferation due to the secretion of factors enhancing proliferation. In light of these considerations, this experiment did not clarify a potential influence of PTPNS1 or CD47 ectodomain shedding on neural migration or differentiation. Purified PTPNS1 and CD47 added to neurosphere cultures would be necessary to determine whether such an effect takes place.

3.4 Imprinting and neural progenitor cells

Two other differentially expressed genes were *mesoderm specific transcript* (*Mest*/*Peg1*) and *paternally expressed gene 3* (*Peg3*), both of which were down-regulated during differentiation by about two-fold. These genes are known to be imprinted. Imprinting is defined as a differential expression of two alleles of a gene depending on whether the allele was maternally or paternally inherited. Paternal (or maternal) imprinting means that an allele inherited from the father (or mother) is not expressed in the offspring. In case of *Mest* and *Peg3*, the maternal alleles are imprinted by CpG island methylation and therefore silenced during development. Only the paternal alleles of these genes remain active (Riesewijk et al., 1997; Lefebvre et al., 1997; Li et al., 2000). The allele-specific gene expression is ensured by methylation of CpG islands in the promoter region. However, the methylation and expression of imprinted genes can be unstable in cultured cells (Schumacher and Doerfler, 2004; and references therein).

Peg3 encodes a C2H2-type zinc finger protein that is synthesized in embryonic and adult brain. Female mice with mutated *Peg3* display retarded growth as well as a striking impairment of maternal behavior that frequently results in death of the offspring (Li et al., 1999).

Mest is widely expressed in the mouse embryo, especially in mesoderm-derived tissues. Loss of *Mest* function is associated with two distinct phenotypes: intrauterine growth retardation and abnormal maternal response to newborns (Lefebvre et al., 1998). The function of the encoded protein is unknown, but sequence similarities suggest that it might have a hydrolytic activity. Both alleles of *Mest* are active in embryonic day four blastocyst cells. In later stages of development and in all adult tissues tested, however, only expression of the paternal allele was found by RT-PCR using RNA from tissue homogenates (Reule et al., 1998). The human *Mest* gene also undergoes maternal imprinting. Only the paternal allele was found to be expressed in a number of fetal tissues (Riesewijk et al., 1997). However, this study described biallelic expression of *Mest* in human adult blood lymphocytes, but suggested that in these cells the imprint is probably lost during development instead of not being established at all. In fetal and adult blood from mice, the imprint is established (Riesewijk et al., 1997).

Since expression of these genes was down-regulated during the differentiation of progenitor cells by about two-fold, it was tempting to speculate about a biallelic expression in neural progenitors and a loss of expression from one allele in differentiated cells. To test whether the imprinting pattern was present in the adult neural progenitors, a restriction fragment length polymorphism test described by Kaneko-Ishino et al. was applied (1995). This test makes use of a strain-specific sequence variation in the *Mest* gene. Neurospheres were generated from hybrid mice that stemmed from mating a female *Mus musculus* with a male *Mus spretus*. A fragment of the *Mest* transcript from undifferentiated and differentiated neurosphere cells was reverse-

transcribed and amplified by RT-PCR and the resulting cDNA was digested with *Taq*1 to determine which allele was expressed in the cells. Only the paternal allele was detected in both undifferentiated and differentiated neurosphere cells (Fig. 26). This demonstrates that the imprint is present in the cultured neural progenitor cells, at least in cells from postnatal day eight mice after three weeks in culture, and does not change during differentiation. Thus, down-regulation of this gene can not be explained by a change from biallelic to monoallelic expression, but results from a lower expression of the single parentally expressed allele.

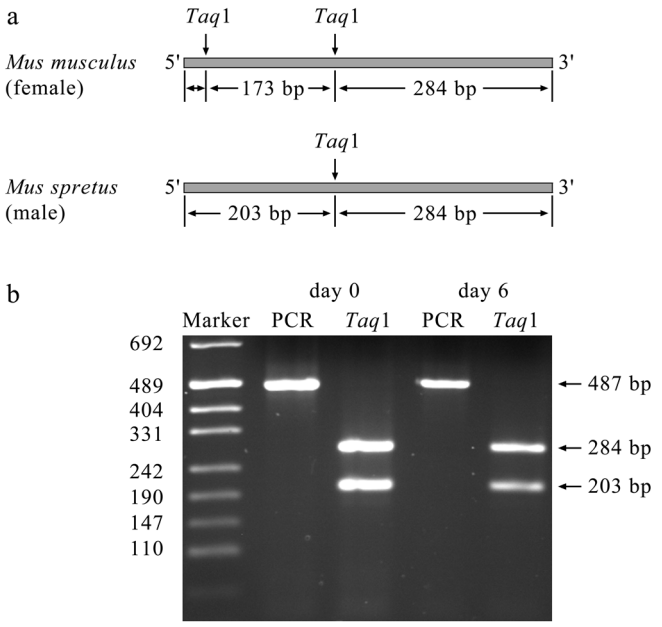

Figure 26. (a) Verification of *Mest* imprinting in neurosphere cells from interspecies mice by restriction fragment length polymorphism analysis. *Taq*1 restriction sites in a fragment of *Mest* cDNA are shown. The fragment contains two such sites in the *Mus musculus* variant and one in the *Mus spretus* variant. (b) RNA was prepared from undifferentiated and differentiated neurospheres from interspecies mice with the maternal allele from *Mus musculus* and the paternal allele from *Mus spretus*. The fragments of the *Mest* transcripts were reverse-transcribed and amplified by RT-PCR and the cDNA was digested with *Taq*1. Separation of undigested and digested PCR products by 1.5% agarose gelelectrophoresis revealed that in both cell types only the paternal allele was detectable. Numbers on the left correspond to the molecular weight marker bands.

3.5 Molecules relevant for adult neural progenitor cells

Proliferation, migration, and differentiation of the progenitor cells have to be regulated properly. The mechanisms regulating these properties are probably mediated by a number of molecules. The microarray analysis revealed many genes that might play a role in these regulatory processes. Among these are three down-regulated genes, whose products are secreted factors: *heparin-binding EGF-like growth factor* (*Hb-Egf*), *neural epidermal growth factor-like-like 2* (*Nell2*), and *pleiotrophin* (*Ptn*). Another down-regulated gene whose product has been implicated in neural morphogenic processes is *tenascin C*, an extracellular matrix glycoprotein. In addition, *brain fatty acid binding protein*, three genes related to insulin-like growth factor signaling, and a number of transcription factors changed in expression. The down-regulation of

Hb-Egf and *Nell2* was verified by semi-quantitative RT-PCR (Fig. 16). In the following, selected genes whose function presumably relates to communication, maintenance, and differentiation *in situ* are presented. Additional data about the *in vitro* and *in situ* localization, and about the expression of potential interacting factors is given as well.

3.5.1 Pleiotrophin

The transcript for pleiotrophin (PTN), a secreted cytokine, decreased up to four-fold during differentiation. Expression of *syndecan-3*, the gene for a membrane-bound PTN receptor, also decreased in the experiment (Tab. 1). The down-regulation of *pleiotrophin* was confirmed by semi-quantitative RT-PCR (Fig. 16). Immunostainings of neurosphere cells revealed that PTN was restricted to compact cells in the center of the neurosphere and disappeared as cells dissociated from the sphere and differentiated (Fig. 27). Immunohistofluorescence analysis revealed that in the rostral migratory stream, cytoplasmic PTN immunoreactivity was associated with small vessels (arrow in Fig. 28 a), as has been described before (Yeh et al., 1998). PSA-NCAM-positive neuroblasts did not produce PTN (Fig. 28 a). In the posterior SVZ, PTN-positive cells appeared to co-synthesize HUC/D (Fig. 28 c arrowhead), which is a neuronal marker, and CD24 (Fig. 28 d), which is present in ependymal cells and type A neuroblasts (Calaora et al., 1996). PTN immunoreactivity was also co-detected in the same cell layer as the glial marker GFAP in the anterior SVZ (Fig. 28 b), but because of the tight association of the glial processes with neuroblasts and ependymal cells in this layer, only electron microscopic analysis can unambiguously ascertain whether PTN is expressed by glial cells. Nuclear PTN staining in the superior-posterior ventricle wall (Fig. 28 b-d) was mostly punctate (arrow in Fig. 28, d), whereas more homogeneous nuclear staining was characteristically seen in other regions of the SVZ. It is worth noting here, that PTN has previously been shown to bind to nucleolin, a major nucleolar protein, that functions as a shuttle protein between the nucleus and cytoplasm (Take et al., 1994). In addition, distinct PTN-positive spots were found in the anterior wall abutting the striatum, the most anterior portion of the superior ventricle wall as well as in its rostral extension (arrowheads in Fig. 28 a, e). These spots appeared cytosolic and were difficult to assign to specific cell types. In summary, the secreted cytokine PTN is synthesized by immature proliferating progenitors *in vitro*. The *pleiotrophin* transcript and the encoded protein decreased during differentiation *in vitro*. *In situ*, PTN seemed to be present in SVZ progenitor cells and/or in immature descendants.

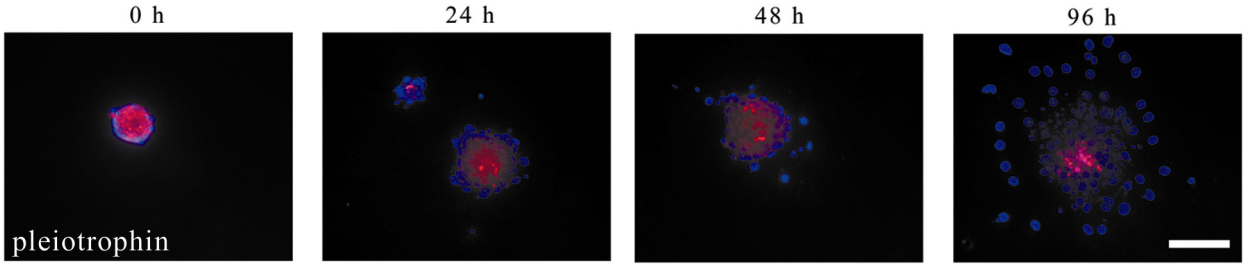

Figure 27. Immunofluorescence microscopy of undifferentiated neurospheres and of cells at 24 h, 48 h, and 96 h after induction of differentiation. Cells were immunolabeled with an antibody against pleiotrophin. Nuclei are stained with DAPI (blue). Scale bar equals 100 µm.

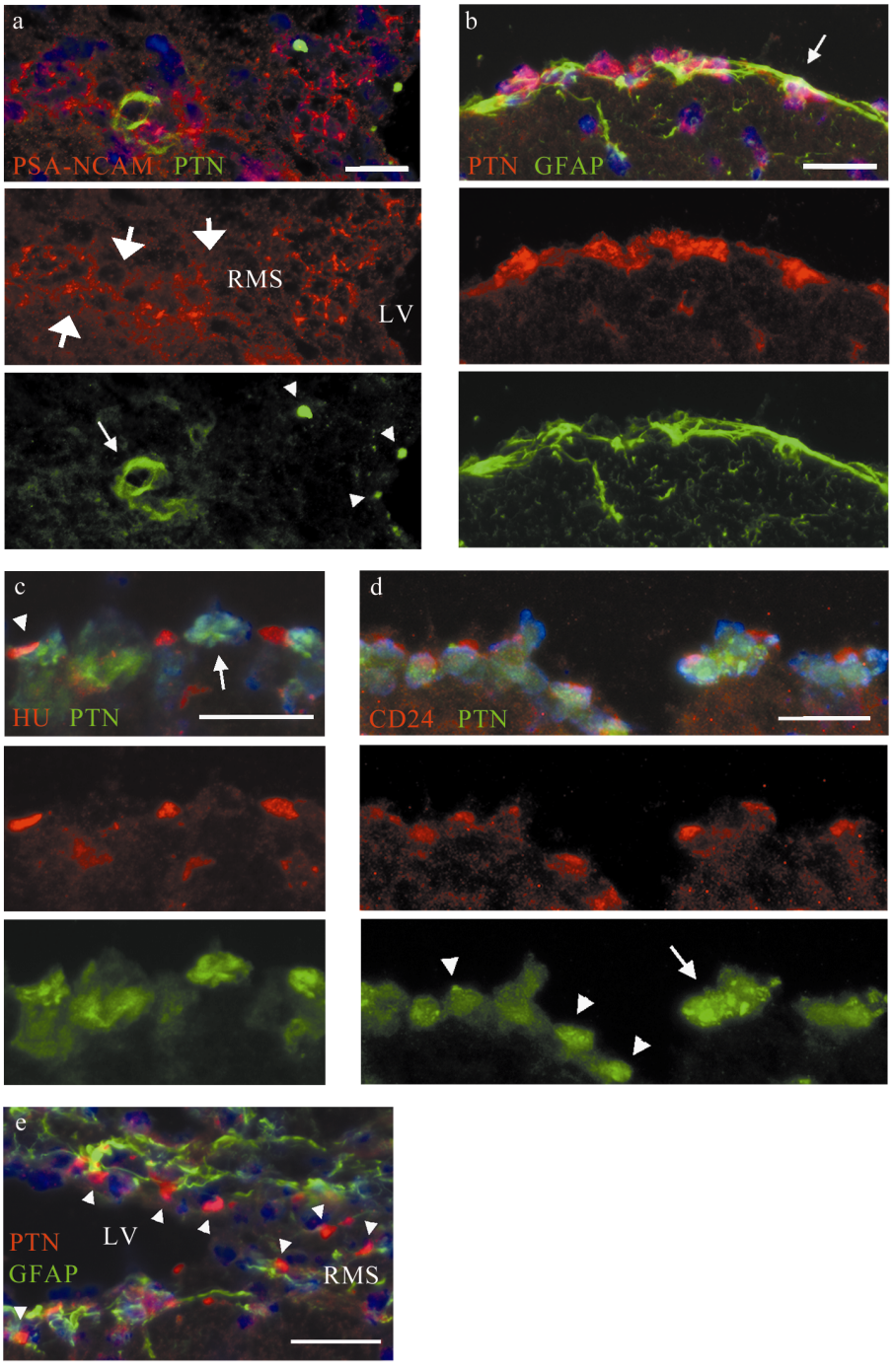

Figure 28. Localization of pleiotrophin (PTN) on sagittal adult brain sections as determined by immunofluorescence microscopy. A nuclear staining of the PTN antibody can be seen in cells of the lateral ventricle wall (red signal in b, green signals in c, d). Green signals in b: GFAP. Red signals in c: HUC/D. Red signals in d: CD24. Arrow in b points at a GFAP-positive cell. Arrow in c points at a HUC/D-negative cell. Arrowheads in a, e point at a spot-like PTN pattern. a: green signals PTN, red signals PSA-NCAM. e: green signals GFAP, red signals PTN. In the RMS, the PTN antibody stains small vessels (single small arrow pointing at green signals in a. Thick arrows in a indicate PSA-NCAM-positive cells in the RMS. Scale bars equal 50 µm.

3.5.2 Brain fatty acid binding protein

The gene coding for brain fatty acid-binding protein (FABP7/BLBP) was found to be down-regulated two- to three-fold by the cells after induction of differentiation. FABP7 is a brain-specific member of the FABP family of cytosolic proteins that bind small hydrophobic signaling molecules. The spatiotemporal production of mouse *Fabp7* mRNA and protein correlates with differentiation of neuronal and glial cells (Feng et al., 1994; Kurtz et al., 1994). To analyze the presence and localization of FABP7 in neurosphere cells and in the SVZ and RMS, immuno-fluorescence and immunohistofluorescence analyses were performed. Antibodies against FABP7 stained the neurosphere as well as the cell body and cellular processes of differentiating cells close to the cell cluster (Fig. 29). In the brain, FABP7 was found in the SVZ and RMS. The FABP7 signals coincided entirely with the GFAP staining, which strongly suggests that SVZ astrocytes synthesize FABP7 (Fig. 30). Since the monoclonal GFAP antibody used is known to specifically recognize type B cells (neurogenic astrocytes), but not type C cells (transit ampli-fying cells) (Doetsch et al., 2002), this demostrated FABP7 expression by type B cells. This specific localization in the SVZ and presumed function of FABP7 in signaling strongly indicates that FABP7 has an important role in adult neurogenesis and places it among the most interesting candidate genes for further studies.

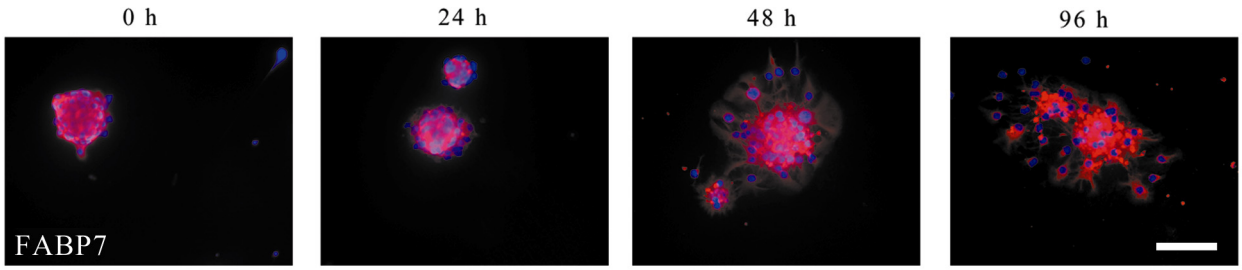

Figure 29. Immunofluorescence microscopy of undifferentiated neurospheres and of cells at 24 h, 48 h, and 96 h after induction of differentiation. Cells were immunolabeled with an antibody against FABP7. Nuclei were stained with DAPI (blue). Scale bar equals 100 µm.

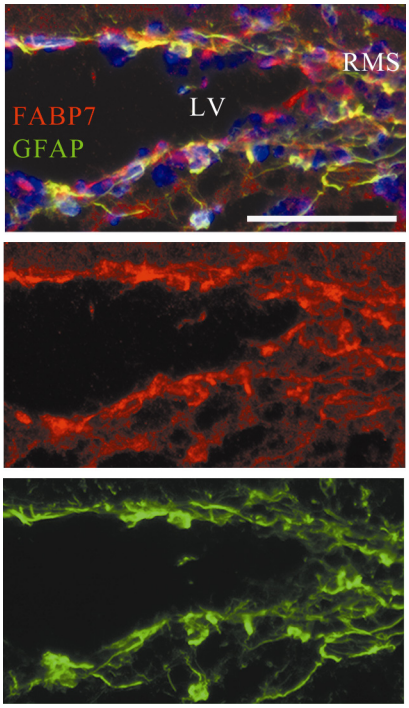

Figure 30. Colocalization of FABP7 (red) and GFAP (green) in the SVZ and RMS on sagittal adult brain sections as determined by immunofluorescence microscopy. Blue signals: DAPI-stained nuclei. LV: lateral ventricle. RMS: rostral migratory stream. Scale bar equals 50 µm.

3.5.3 Insulin-like growth factor binding proteins

Drastic changes in the expression of three genes coding for *insulin-like growth factor binding proteins* (*Igfbp*) were found. At day four *Igfbp2* was down-regulated 16-fold in the BDNF series and 26-fold in the NT4 series. *Igfbp4* was down-regulated about nine-fold in both series. In contrast, transcript levels of *Igfbp5* increased four-fold in both series (Tab. 1). The decrease in *Igfbp2* expression was verified by RT-PCR (Fig. 16).

Insulin-like growth factor 1 (IGF1) is a neurotrophic factor known to play an important role in promoting cell proliferation and differentiation during normal brain development and maturation. IGF1 activity is regulated by IGF binding proteins, the IGFBPs. Since the expression of *Igfbps* changed so drastically, it was further investigated whether the genes encoding their substrate IGF1 or the two IGF1 receptors, the insulin receptor and the IGF1 receptor, are expressed in neural progenitor cells. In addition, it was tested whether this expression changed during differentiation. It turned out that *Igf1* was expressed by the neural progenitor cells (Fig. 31). The transcript level decreased during differentiation of the cells. The genes for the *Igf1 receptor* and the *insulin receptor* were also expressed by the progenitor cells (Fig. 31). If at all, these transcripts only slightly decreased. For a discussion of these results, see section 4.3.3.

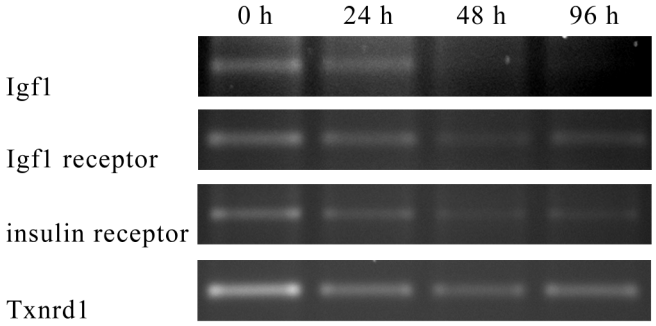

Figure 31. Expression of *Igf1*, *Igf1 receptor*, and *insulin receptor* during neural progenitor cell differentiation. Relative gene expression levels before and after induction of differentiation are visualized by semi-quantitative RT-PCR analysis. Expression of *Txnrd1* was used as a reference.

3.5.4 Transcriptional regulators

Changes in the transcriptome are controlled by DNA binding proteins, by proteins associated with the transcriptional machinery, or by other components of the chromatin. Genes that were differentially regulated during the differentiation of neural progenitors were listed in the 'chromatin-associated components and nuclear factors' category (Tab. 1). Interestingly, for most genes in this category the changes in transcript levels are moderate, rarely exceeding three-fold.

Among the transcription factors with relevant expression changes were five that decreased in expression. Three of these, *transcription factor 12* (*Htf4*/*Heb*/*Reb*), *basic helix loop helix containing domain class B2* (*Bhlhb2*/*Stra13*/*Clast5*/*Sharp2*/*Dec1*), and *oligodendrocyte transcription factor 2* (*Olig2*), belong to the helix-loop-helix class. Increasing mRNA levels of eight transcription factors were identified. These include four genes encoding for zinc finger proteins related to transcription: the *RIKEN cDNA 3110024A21 gene*, *ENSMUSG00000013419*, and the genes encoding ZDHHC9 and DAN (neuroblastoma suppressor of tumorigenicity 1). Furthermore, the bHLH-leucine zipper transcription factor *sterol regulatory element binding factor 1*, the forkhead transcriptional activator *Foxj1* (*Hfh-4*/*Fkhl13*/*Hnf-3*), and *inhibitor of DNA binding 4* (*Id4*), which is a negative regulator of helix-loop-helix transcription factors, were up-regulated. The transcript level increase of *Id4* was confirmed by semi-quantitative RT-PCR (Fig. 16).

Among the down-regulated genes encoding for chromatin and chromatin-associated proteins were *histone 2Az* (*H2A.Z*), *histone 2Ax* (*H2A.X*), the high mobility group gene *Hmgb2* (formerly *Hmg2*), and *thymopoietin* (*Lap2*). Transcript levels of *histone 2B* (*H2B*) increased. The down-regulation of *H2A.Z* was confirmed by semi-quantitative RT-PCR (Fig. 16). The finding of an altered expression of *H2A.X* has to be looked at with caution, since the coding sequence of this gene differs only slightly from the ten *H2A1* and the single *H2A2* variants, in contrast to *H2A.Z*, which differs considerably. Therefore, a cross-hybridization of *H2A1*, *H2A2* and *H2A.X* transcripts could have occured.