Short Communication

Prolactin Increases Expression of Cytoskeletal Proteins in SK-N-SH Cells

(prolactin / nestin / microtubule-associated protein 2)

T. HAVRÁNEK[1], Z. BAČOVÁ[1,2], V. ŠTRBÁK[1,2], Z. LEŠŤANOVÁ[1], J. BAKOŠ[1,3]

[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Department of Normal and Pathological Physiology, Faculty of Medicine, Slovak Medical University in Bratislava, Slovakia
[3]Institute of Physiology, Faculty of Medicine in Bratislava, Comenius University in Bratislava, Slovakia

Abstract. Although many studies have demonstrated the role of prolactin in the central nervous system, there is a considerable lack of known effects of prolactin on the parameters of neurogenesis and neuronal differentiation. The aim of the present study was to test whether prolactin changes gene expression and protein levels of nestin and microtubule-associated protein 2 (MAP2) in neuroblastoma (SK-N-SH) and glioblastoma (U-87MG) cells. Nestin and MAP2 represent cytoskeletal proteins associated with neuronal differentiation and they contribute to radial growth of the axons, dendrites and glial processes. SK-N-SH and U-87MG cells were exposed to prolactin (10 nM) for 48 h. Total mRNA was extracted. After reverse transcription, qPCR with specific primers for nestin and MAP2 was performed. The levels of proteins were measured by the In-Cell Western assay. Mitochondrial activity test was used to evaluate the viability of cells under the influence of prolactin. Incubation with 10 nM prolactin did not change the viability, either in SK-N-SH or in U-87MG cells. Prolactin significantly increased the gene expression and protein levels of both nestin and MAP2 in SK-N-SH cells, while no significant changes were observed in U-87MG cells. The presented data suggest that prolactin is linked to the regulation of cytoskeletal proteins in the neuronal type of cells and might be important for their differentiation.

Received May 15, 2014. Accepted June 9, 2014.

The work was supported by project 2/0132/12 of the Grant Agency of the Ministry of Education and Slovak Academy of Sciences (VEGA), and by the Slovak Research and Development Agency project APVV-0253-10.

Corresponding author: Ján Bakoš, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, Bratislava, Slovakia. Phone: (+421) 2-54772800; Fax: (+421) 2-54774908; e-mail: j.bakos@savba.sk

Abbreviations: ICW – In-Cell Western assay, MAP2 – microtubule-associated protein 2, PBS – phosphate-buffered saline, RT – room temperature.

Introduction

Prolactin is an important protein hormone secreted primarily from lactotroph cells of the pituitary gland. The tertiary protein structure of prolactin is closely related to growth hormone and placental lactogen (Forsyth and Wallis, 2002), implicating its role in the regulation of metabolic and growth processes. Although prolactin is known for its function in mammary gland development, recently it was shown that exogenously applied, it affects proliferation of neuronal stem cells (Pathipati et al., 2011). Furthermore, high expression of prolactin receptors in the brain areas (subventricular zone) related to neurogenesis was found (Roky et al., 1996). Prolactin can increase hippocampal precursor cell numbers both *in vitro* and *in vivo* (Walker et al., 2012). It was previously found that prolactin mediates enhanced olfactory neurogenesis in mice (Mak and Weiss, 2010). *In vitro*, prolactin was associated with cell cycle regulation (Arumugam et al., 2011) and might be considered as a growth and differentiating factor (Brooks, 2012). Anti-apoptotic effects of prolactin were described in SH-SY5Y neuroblastoma cells (Um and Lodish, 2006). Some studies demonstrated that prolactin is involved in the regulation of cultured rat astrocyte mitogenesis (DeVito et al., 1993) and increases human glioma growth and survival (Ducret et al., 2002).

One of the important intermediate filament proteins is nestin, a major component of the developing cytoskeleton in radial glial cells, germinal matrix cells (Lendahl et al., 1990), as well as adult neural precursors (Hockfield and McKay, 1985; Blumcke et al., 2001). Nestin participates in key cell processes, such as proliferation, migration and cell survival (Ehrmann et al., 2005; Means et al., 2005; Jing et al., 2012). Another neuronal cytoskeletal component is microtubule-associated protein 2 (MAP2), which takes part in maintaining the cellular architecture and internal organization (Yamaguchi et al.,

2008). MAP2 protein is known for its microtubule-stabilizing activity and for regulating microtubule networks in the dendrites of neurons, resulting in dendrite elongation (Harada et al., 2002). The ability of MAP2 to interact with microtubules might be critical for neuromorphogenic processes, such as cell division, neuronal migration and neurite extension, during which networks of microtubules are reorganized in a coordinated manner (Dehmelt and Halpain, 2005).

Whether prolactin affects cytoskeletal proteins related to differentiation of neuronal cells is not clear. To extend our knowledge on this issue, we tested whether incubation of neuroblastoma (SK-N-SH) and glioblastoma (U-87 MG) cells in the presence of prolactin results in increased gene and protein expression of both nestin and MAP2. Mitochondrial activity test was used to evaluate the viability of cells under the influence of prolactin.

Material and Methods

Cell cultures

Human neuroblastoma SK-N-SH (80th passage) and glioblastoma U-87MG (180th passage) cell lines obtained from the American Type Culture Collection (ATCC, LGC Standards, Lomianki, Poland) were cultured in Dulbecco's Minimum Essential Medium supplemented with 1 % L-glutamine, 0.1 % non-essential amino acids, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 % foetal bovine serum MYCOPLEX (all from PAA, Pasching, Austria) in an incubator in humidified atmosphere containing 5 % of CO_2 at 37 °C. As the cells became confluent, they were passaged by gentle trypsinization.

Prolactin treatment

Cells were incubated in the presence of 10 nM prolactin (human recombinant prolactin, Biochrom AG, Berlin, Germany) for 48 h. Lower (5 nM) and also higher (100 nM) doses of prolactin applied *in vitro* could be found in the literature (Van Coppenolle et al., 2004; Yang et al., 2013). In the present study, the medium dose and prolonged time of incubation was selected. Control samples were incubated in basal medium. Medium with or without prolactin was changed every 24 h.

RNA isolation, reverse transcription and qPCR

Total RNA was extracted using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. The quantity, integrity and purity of RNA were determined by a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc, Rockford, IL). RNA (1 µg) was reversely transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Bratislava, Slovakia) according to the manufacturer's protocol in a volume of 20 µl by incubation at 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, followed by a hold at 4 °C. First strand cDNA was stored at -20 °C until use. Quantitative RT-PCR (qPCR) was performed in the AB7900 instrument (Life Technologies) using Power SYBR Green PCR Master Mix (Life Technologies).

The reaction volume was 20 µl containing master mix, 0.3 µM each of forward and reverse primer (GAPDH: fw-CGTGGAAGGACTCATGAC, rw-CAATTCGTTGTCATACCAG (Choi et al., 2011), nestin: fw-GAGAGGGAGGACAAAGT CCC, rw-TCCCTCAGAGACTAGCGCAT (Hao et al., 2003), MAP2: fw-TCAGAGGCAATGA CCTTACC, rw-GTGGTAGGCTCTTGGTCTTT (Nitti et al., 2010)), 2 µM ROX reference dye, and 10 ng of cDNA template. PCR was performed for 40 cycles according to the following protocol: activation of the Taq polymerase (Life Technologies) at 95 °C for 10 min, followed by 40 cycles of denaturing at 95 °C for 15 s, annealing/extension at 60 °C for 1 min followed by fluorescence measurement (SYBR Green and ROX, respectively). To identify the reaction products, melting curve analysis was performed. Relative mRNA expression was calculated by the Livak method (Livak and Schmittgen, 2001) for relative gene expression analysis with $2^{-\Delta\Delta Ct}$ comparative threshold.

MTT assay

Evaluation of cell viability was performed using the MTT assay, which represents a technique for measuring cell mitochondrial activity. The mitochondrial succinate-tetrazolium reductase system converts yellow tetrazolium MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, St. Louis, MO) to purple formazan. Cells ($2x10^5$) were seeded in 100 µl media (with or without prolactin). After 48 h, cells were incubated for 1 h with 50 µl of MTT solution (1 mg/ml in phosphate-buffered saline (PBS)). Formazan was dissolved in 150 µl of dimethyl sulphoxide (DMSO, Sigma-Aldrich). The absorbance was measured at 570 nm with an Epoch Microplate Spectrophotometer (Biotek, Bad Friedrichshall, Germany).

In-Cell Western Assay

Protein immunodetection was performed using specific rabbit monoclonal antibodies against nestin and MAP2 (Sigma-Aldrich) in 1 : 500 dilution in PBS. As the housekeeper, GAPDH was detected by mouse monoclonal anti-GAPDH antibody (Sigma-Aldrich, 1:500). Specific rabbit antibodies were detected with the goat anti-rabbit (800CW) secondary antibody, while the mouse anti-GAPDH primary antibody was reacted with goat anti-mouse (680CW) secondary antibody.

In-Cell Western (ICW) assay (Sigma-Aldrich) was performed in 96-well cultivation test plates (TPP, Trasadingen, Switzerland). Cells, at a density of $6–7\times10^3$ per well, were incubated with or without 10 nM prolactin. After 48 h cells were washed with 150 µl cold PBS solution and then fixed with 150 µl cold 4% paraformaldehyde for 15 min at room temperature (RT). Cells were permeabilized by twice washing with 200 µl PBS + 0.1 % Triton X-100 for 5 min per wash at RT with mild shak-

ing. The plate was incubated with 20 µl of specific monoclonal antibodies for 3 h at RT with mild shaking. As a negative control, PBS was used. After incubation with first antibody, the plate was washed 3-times in PBS + 0.1% Tween 20, at RT for 5 min per wash with mild shaking. The plate was then incubated with 20 µl of secondary antibodies for 2 h at RT. Plates were washed twice with PBS and 50 µl PBS was added for the measurement. The intensity of ICW reaction was measured with the LI-COR Odyssey Near Infrared Imaging System (LI-COR, Lincoln, NE). The background was set up with IRDye® secondary antibodies only (both 680CW and 800CW) and no primary antibodies. Quantification was performed with the corresponding software LI-COR Odyssey® Software Image studio 2 (LI-COR), scan resolution of 169 µm, an offset focus of 3.5 mm and initial intensities of 4 for 680CW (red) and 800CW (green) channels. The measured protein levels were quantified based on the ratio of green to red intensities within the same well. They were expressed as the percentage increase in green to red fluorescence ratio as compared to the controls.

Statistics

The data from the MTT test and ICW assay are expressed as means ± SEM. Statistical comparisons were performed using Student's *t*-test. The value of $P < 0.05$ was considered statistically significant.

The data of gene expression analyses are expressed as a ratio of the target gene in the test sample (cells with prolactin) to the calibrator sample (cells in basal medium), normalized to the expression of the reference gene – *GAPDH*. The baseline expression level of the control group was set to 1. Data from test samples were compared to calibrator samples and differences were considered significant at the level of $P < 0.05$ using Student's *t*-test. The results are shown as means ± SEM.

Results and Discussion

In the present study, we found that prolactin increases the levels of both cytoskeletal proteins in the neuronal type of cells, while no such effect was observed in the glial/astrocyte type of cells. Incubation with prolactin did not change the viability, either in neuroblastoma SK-N-SH cells (106.57 ± 2.88 % compared to control 100 ± 4.7 %, N = 10), or in glioblastoma U-87MG cells (96.26 ± 1 % compared to control 100 ± 1.53 %, N = 10). Although prolactin was proved to play a role in the regulation of cell cycle in primary β-cells (Arumugam et al., 2011) and it is considered as a growth and differentiating factor in various cells (Brooks, 2012), in our study no effect of prolactin on cell viability was observed. In different models, prolactin was shown to increase viability of prostate tumour cells *in vitro* (Dagvadorj et al., 2007) and it was suggested that prolactin might act as a survival factor for prostate epithelium (Ahonen et al., 1999). However, its role in neuronal cell proliferation remains unclear. At least with the present dose of prolactin, there was no pro-proliferative response of the cells. On the other hand, the effect of prolactin on the components of cytoskeleton resulted in major changes. In SK-N-SH cells, 48 h incubation with prolactin significantly increased both nestin (Fig.1) and

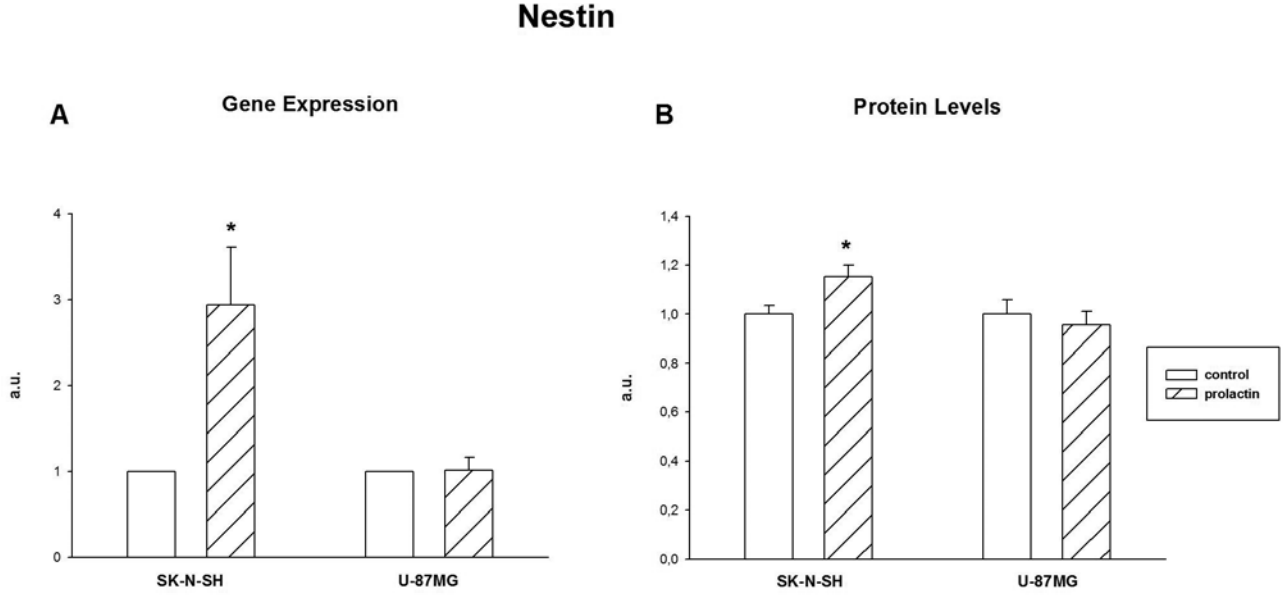

Fig. 1. Effects of prolactin on mRNA (**A**) and protein (**B**) levels of nestin in neuroblastoma (SK-N-SH) and glioblastoma (U-87MG) cells. Cells were incubated with or without 10 nM prolactin for 48 h. Gene expression data are expressed as relative mRNA expression calculated by $2^{-\Delta\Delta Ct}$ by the Livak method (Livak and Schmittgen, 2001). Relative changes in protein levels were measured by In-Cell Western Blot, where GAPDH served as an endogenous control**.** Means are represented as bars ± SEM (N = 6). Significant changes marked with * $P < 0.05$ compared to control cells.

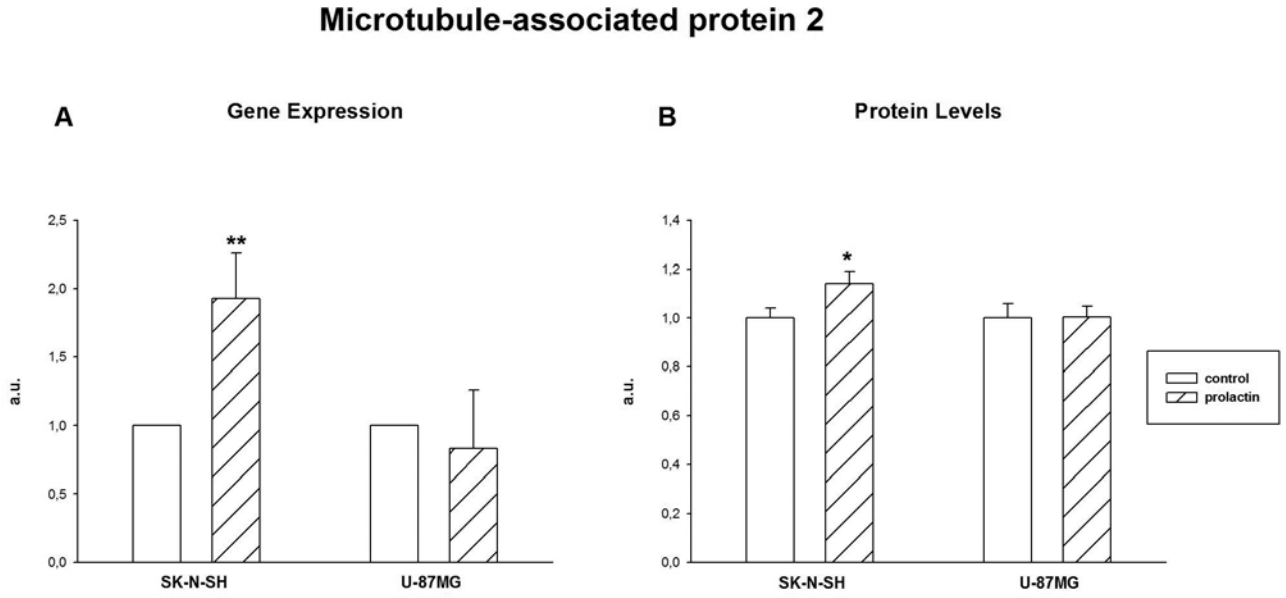

Fig. 2. Effects of prolactin on mRNA (**A**) and protein (**B**) levels of microtubule-associated protein-2 (MAP2) in neuroblastoma (SK-N-SH) and glioblastoma (U-87MG) cells. Cells were incubated with or without 10 nM prolactin for 48 h. Gene expression data are expressed as relative mRNA expression calculated by $2^{-\Delta\Delta Ct}$ by the Livak method (Livak and Schmittgen, 2001). Relative changes in protein levels were measured by In-Cell Western Blot, where GAPDH served as an endogenous control**.** Means are represented as bars ± SEM (N = 6). Significant changes marked with * $P < 0.05$, ** $P < 0.01$ compared to control cells.

MAP2 (Fig. 2) gene expression and protein levels, while no significant changes were observed in U-87MG cells. Despite that U87-MG cells naturally express the prolactin receptor (Ducret et al., 2002), no effect on the expression of cytoskeletal proteins suggests lower sensitivity of astrocytes and a cell-specific effect. Different signalling pathways would substantiate such result in the glioblastoma cell line. Nestin is expressed in a variety of undifferentiated tissues under normal and pathological conditions (Wiese et al., 2004; Krupkova et al., 2010), but it is also considered as a stem/progenitor cell marker in a variety of differentiated tissues, for example in the mammalian skin or pancreas (Toma et al., 2001; Kim et al., 2004).

Our results suggest that prolactin might contribute to the differentiation of neuronal cell lineages, which corresponds with one recent study on a population of pituitary cells (Weiss et al., 2009). Weiss et al. (2009) showed that stimulation of the prolactin-expressing phenotype of cells was associated with the expression of nestin. Another study has indicated that prolactin given in a high dose induces neuron differentiation in mice (Khodr et al., 2009) and there is evidence that elevated prolactin during pregnancy increases neurogenesis in rodents (Larsen and Grattan, 2012). In our *in vitro* model we observed an increase of MAP2 in SK-N-SH cells at both gene and protein levels. This supports the neurotrophic potential of prolactin for neurons. As MAP2 is important for the regulation of microtubule networks in the dendrites of neurons, the role of prolactin in the neurite outgrowth might be speculated. Indeed, in one study it was observed that prolactin promotes neurite extension (Chalisova et al., 1991). Our results suggest that prolactin modulates cytoskeletal proteins in the neuronal type of cells, which might be especially important for their differentiation in early postnatal development.

References

Ahonen, T. J., Härkönen, P. L., Laine, J., Rui, H., Martikainen, P. M., Nevalainen, M. T. (1999) Prolactin is a survival factor for androgen-deprived rat dorsal and lateral prostate epithelium in organ culture. *Endocrinology* **140**, 5412-5421.

Arumugam, R., Fleenor, D., Lu, D., Freemark, M. (2011) Differential and complementary effects of glucose and prolactin on islet DNA synthesis and gene expression. *Endocrinology* **152**, 856-868.

Blumcke, I., Schewe, J. C., Normann, S., Brustle, O., Schramm, J., Elger, C. E., Wiestler, O. D. (2001) Increase of nestin-immunoreactive neural precursor cells in the dentate gyrus of pediatric patients with early-onset temporal lobe epilepsy. *Hippocampus* **11**, 311-321.

Brooks, C. L. (2012) Molecular mechanisms of prolactin and its receptor. *Endocr. Rev*. **33**, 504-525.

Chalisova, N. I., Mel'kishev, V. F., Akoev, G. N., Liudyno, M. I., Kurenkova, T. (1991) The stimulating effect of prolactin on the neurite growth of sensory neurons in an organotypic culture. *Tsitologiia* **33**, 29-31. (in Russsian)

Choi, S. A., Hwang, S. K., Wang, K. C., Cho, B. K., Phi, J. H., Lee, J. Y., Jung, H. W., Lee, D. H., Kim, S. K. (2011) Therapeutic efficacy and safety of TRAIL-producing hu-

man adipose tissue-derived mesenchymal stem cells against experimental brainstem glioma. *Neuro. Oncol*. **13**, 61-69.

Dagvadorj, A., Collins, S., Jomain, J. B., Abdulghani, J., Karras, J., Zellweger, T., Li, H., Nurmi, M., Alanen, K., Mirtti, T., Visakorpi, T., Bubendorf, L., Goffin, V., Nevalainen, M. T. (2007) Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. *Endocrinology* **148**, 3089-3101.

Dehmelt, L., Halpain, S. (2005) The MAP2/Tau family of microtubule-associated proteins. *Genome. Biol.* **204**, 1-10.

DeVito, W. J., Avakian, C., Stone, S., Okulicz, W. C. (1993) Prolactin-stimulated mitogenesis of cultured astrocytes is mediated by a protein kinase C-dependent mechanism. *J. Neurochem.* **60**, 832-842.

Ducret, T., Boudina, S., Sorin, B., Vacher, A.M., Gourdou, I., Liguoro, D., Guerin, J., Bresson-Bepoldin, L., Vacher, P. (2002) Effects of prolactin on intracellular calcium concentration and cell proliferation in human glioma cells. *Glia* **38**, 200-214.

Ehrmann, J., Kolar, Z., Mokry, J. (2005) Nestin as a diagnostic and prognostic marker: immunohistochemical analysis of its expression in different tumours. *J. Clin. Pathol.* **58**, 222-223.

Forsyth, I. A., Wallis, M. (2002) Growth hormone and prolactin-molecular and functional evolution. *J. Mammary Gland Biol. Neoplasia* **7**, 291-312.

Hao, H. N., Parker, G. C., Zhao, J., Barami, K., Lyman, W. D. (2003) Differential responses of human neural and hematopoietic stem cells to ethanol exposure. *J. Hematother. Stem Cell Res.* **12**, 389-399.

Harada, A., Teng, J., Takei, Y., Oguchi, K., Hirokawa, N. (2002) MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. *J. Cell. Biol.* **158**, 541-549.

Hockfield, S., McKay, R. D. (1985) Identification of major cell classes in the developing mammalian nervous system. *J. Neurosci.* **5**, 3310-3328.

Jing, Y. H., Hou, Y. P., Song, Y. F., Yin, J. (2012) Methylprednisolone improves the survival of new neurons following transient cerebral ischemia in rats. *Acta Neurobiol. Exp. (Wars.)* **72**, 240-252.

Khodr, C. E., Hurley, D. L., Phelps, C. J. (2009) Prolactin induces tuberoinfundibular dopaminergic neuron differentiation in Snell dwarf mice if administered beginning at 3 days of age. *J. Neuroendocrinol.* **21**, 558-567.

Kim, S. Y., Lee, S. H., Kim, B. M., Kim, E. H., Min, B. H., Bendayan, M., Park, I. S. (2004) Activation of nestin-positive duct stem (NPDS) cells in pancreas upon neogenic motivation and possible cytodifferentiation into insulin-secreting cells from NPDS cells. *Dev. Dyn.* **230**, 1-11.

Krupkova, O. Jr., Loja, T., Zambo, I., Veselska, R. (2010) Nestin expression in human tumors and tumor cell lines. *Neoplasma* **57**, 291-298.

Larsen, C. M., Grattan, D. R. (2012) Prolactin, neurogenesis, and maternal behaviors. *Brain Behav. Immun.* **26**, 201-209.

Lendahl, U., Zimmerman, L. B., McKay, R. D. (1990) CNS stem cells express a new class of intermediate filament protein. *Cell* **60**, 585-595.

Livak, K. J., Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* **25**, 402-408.

Mak, G. K., Weiss, S. (2010) Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nat. Neurosci.* **13**, 753-758.

Means, A. L., Meszoely, I. M., Suzuki, K., Miyamoto, Y., Rustgi, A. K., Coffey, R. J. Jr., Wright, C. V., Stoffers, D. A., Leach, S. D. (2005) Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. *Development* **132**, 3767-3776.

Nitti, M., Furfaro, A. L., Cevasco, C., Traverso, N., Marinari, U. M., Pronzato, M. A., Domenicotti, C. (2010) PKC δ and NADPH oxidase in retinoic acid-induced neuroblastoma cell differentiation. *Cell. Signal.* **22**, 828-835.

Pathipati, P., Gorba, T., Scheepens, A., Goffin, V., Sun, Y., Fraser, M. (2011) Growth hormone and prolactin regulate human neural stem cell regenerative activity. *Neuroscience* **190**, 409-427.

Roky, R., Paut-Pagano, L., Goffin, V., Kitahama, K., Valatx, J. L., Kelly, P. A., Jouvet, M. (1996) Distribution of prolactin receptors in the rat forebrain. Immunohistochemical study. *Neuroendocrinology* **63**, 422-429.

Toma, J. G., Akhavan, M., Fernandes, K. J., Barnabe-Heider, F., Sadikot, A., Kaplan, D. R., Miller, F. D. (2001) Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.* **3**, 778-784.

Um, M., Lodish, H. F. (2006) Antiapoptotic effects of erythropoietin in differentiated neuroblastoma SH-SY5Y cells require activation of both the STAT5 and AKT signaling pathways. *J. Biol. Chem.* **281**, 5648-5656.

Van Coppenolle, F., Skryma, R., Ouadid-Ahidouch, H., Slomianny, C., Roudbaraki, M., Delcourt, P., Dewailly, E., Humez, S., Crépin, A., Gourdou, I., Djiane, J., Bonnal, J. L., Mauroy, B., Prevarskaya, N. (2004) Prolactin stimulates cell proliferation through a long form of prolactin receptor and K+ channel activation. *Biochem. J.* **377**, 569-578.

Walker, T. L., Vukovic, J., Koudijs, M. M., Blackmore, D. G., Mackay, E. W., Sykes, A. M., Overall, R. W., Hamlin, A. S., Bartlett, P. F. (2012) Prolactin stimulates precursor cells in the adult mouse hippocampus. *PLoS One* **7**, e44371 1-11.

Weiss, S., Siebzehnrübl, F. A., Kreutzer, J., Blümcke, I., Buslei, R. (2009) Evidence for a progenitor cell population in the human pituitary. *Clin. Neuropathol.* **28**, 309-318.

Wiese, C., Rolletschek, A., Kania, G., Blyszczuk, P., Tarasov, K. V., Tarasova, Y., Wersto, R. P., Boheler, K. R., Wobus, A. M. (2004) Nestin expression – a property of multi-lineage progenitor cells? *Cell. Mol. Life Sci.* **61**, 2510-2522.

Yamaguchi, S., Fujii-Taira, I., Murakami, A., Hirose, N., Aoki, N., Izawa, E., Fujimoto, Y., Takano, T., Matsushima, T., Homma, K. J (2008) Up-regulation of microtubule-associated protein 2 accompanying the filial imprinting of domestic chicks (Gallus gallus domesticus). *Brain Res. Bull.* **76**, 282-288.

Yang, N., Liu, C., Peck, A. R., Girondo, M. A., Yanac, A. F., Tran, T. H., Utama, F. E., Tanaka, T., Freydin, B., Chervoneva, I., Hyslop, T., Kovatich, A. J., Hooke, A. J., Shriver, C. D., Rui, H. (2013) Prolactin-Stat5 signaling in breast cancer is potently disrupted by acidosis within the tumor microenvironment. *Breast Cancer Res.* **15**, R73.