Physiol. Res. 60 (Suppl. 1): S83-S91, 2011 *https://doi.org/10.33549/physiolres.932184*

REVIEW

Regulation of the *SOX3* Gene Expression by Retinoid Receptors

G. NIKČEVIĆ[1], N. KOVAČEVIĆ-GRUJIČIĆ[1], M. MOJSIN[1], A. KRSTIĆ[1], †T. SAVIĆ[1], M. STEVANOVIĆ[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Received December 15, 2010
Accepted March 30, 2011
On-line July 19, 2011

Summary

Sox3/SOX3 gene is considered to be one of the earliest neural markers in vertebrates. Despite the mounting evidence that *Sox3/SOX3* is one of the key players in the development of the nervous system, limited data are available regarding the transcriptional regulation of its expression. This review is focused on the retinoic acid induced regulation of *SOX3* gene expression, with particular emphasis on the involvement of retinoid receptors. Experiments with human embryonal carcinoma cells identified two response elements involved in retinoic acid/retinoid X receptor-dependent activation of the *SOX3* gene expression: distal atypical retinoic acid-response element, consisting of two unique G-rich boxes separated by 49 bp, and proximal element comprising DR-3-like motif, composed of two imperfect hexameric half-sites. Importantly, the retinoic acid-induced *SOX3* gene expression could be significantly down-regulated by a synthetic antagonist of retinoid receptors. This cell model provides a solid base for further studies on mechanism(s) underlying regulation of expression of *SOX3* gene, which could improve the understanding of molecular signals that induce neurogenesis in the stem/progenitor cells both during development and in adulthood.

Key words

SOX3 • Retinoic acid • Retinoid receptors • Embryonal carcinoma cells • Neuronal differentiation

Corresponding author

Gordana Nikčević, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, PO BOX 23, 11010 Belgrade, Serbia. Fax: (+) 381 11 3975808. E-mail: gordnik@eunet.rs

Sox3/SOX3 gene

The *Sox/SOX* gene family encodes transcription factors that act as key regulators of diverse developmental processes, such as early embryogenesis, gastrulation, neural induction, formation of various organs and tissues, specification and differentiation of many cell types (Lefebvre *et al.* 2007, Pevny and Lovell-Badge 1997, Wegner 1999). It is known that SOX transcription factors carry a DNA-binding HMG domain and perform their functions in complex interplay with other transcription factors in a manner highly dependent on cell type and promoter context (Kamachi *et al.* 2000). They show both classical and architectural modes of action (Pevny and Lovell-Badge 1997), either activating or repressing specific target genes (Wilson and Koopman 2002). Based on protein sequence comparisons, Sox/SOX transcription factors are divided into 10 distinct groups designated A-J (Bowles *et al.* 2000).

SOX3 together with SOX1 and SOX2 belongs to the SOXB1 subgroup of transcriptional activators (Uchikawa *et al.* 1999), which are pan-neurally expressed and have redundant roles in maintaining the broad developmental potential and identity of neural stem cells (Bylund *et al.* 2003, Pevny and Placzek 2005). In addition to regulating progression of neurogenesis, this group of activators is also operative in post-mitotic neurons (Ekonomou *et al.* 2005, Ferri *et al.* 2004, Rizzoti *et al.* 2004).

Sox3/SOX3 gene is an X-linked member of the family and the closest relative of sex-determining region Y gene (*SRY*) (Stevanovic *et al.* 1993). It is considered to be one of the earliest neural markers in vertebrates,

playing a role in specifying neuronal fate (Brunelli *et al.* 2003).This gene is also implicated in the genetic cascades that direct gonadal development, brain formation, and cognitive function (Pevny and Lovell-Badge 1997, Stevanovic *et al.* 1993, Wegner 1999, Weiss *et al.* 2003). The *Sox3/SOX3* gene is highly expressed in the ventral diencephalon and its deletion is thought to result in either cell death or defects in neuronal activity (Rizzoti *et al.* 2004). Dysfunction of the SOX3 protein disturbs the cellular processes required for cognitive and pituitary developments, leading to mental retardation and growth hormone deficiency in humans (Laumonnier *et al.* 2002, Stankiewicz *et al.* 2005, Woods *et al.* 2005).

Despite the mounting evidence that *Sox3/SOX3* is one of the key players in the development of the nervous system, limited data are available regarding the transcriptional regulation of its expression. Using mouse transgenic reporter assays, Brunelli *et al.* (2003) identified *cis*-regulatory regions of the *mSox3* gene that direct its tissue-specific expression. It has been shown that genomic region comprising 3 kb upstream and 3 kb downstream of the *mSox3* open reading frame, is sufficient to mimic most aspects of the gene's endogenous expression pattern, from the onset of neurogenesis up to midgestation stages (Brunelli *et al.* 2003). The study in *Xenopus laevis* embryos by Rogers *et al.* (2008) indicated that the 1.5 kb upstream regulatory region contains most of the information necessary to recapitulate the spatial and temporal expression of *xSox3* gene. Fine dissection of *xSox3* promoter has revealed the presence of two enhancer regions necessary for full *xSox3* expression, as well as one repression module that restricts the expression of this gene to neuroectoderm (Rogers *et al.* 2008).

Previously we generated data regarding transcriptional regulation of the human *SOX3* gene using embryonal carcinoma (EC) cell line NT2/D1, as a model system. Initially, we determined the *SOX3* transcription start point (*tsp*), promoter region that confers basal promoter activity, as well as positive regulatory elements necessary for optimal promoter activity (Kovacevic Grujicic *et al.* 2005). Also, we found that ubiquitous transcription factors Sp1 (specificity protein 1), USF1 (upstream stimulatory factor 1), NF-Y (nuclear factor Y), MAZ (*myc*-associated zinc finger protein) and PBX1/MEIS1 (pre-B-cell leukaemia homeobox 1/myeloid ecotropic viral integration site 1 homologue) act as positive regulators of *SOX3* gene expression (Krstic *et al.* 2007, Mojsin and Stevanovic 2010, Kovacevic Grujicic *et al.* 2005, Kovacevic Grujicic *et al.* 2008, Stevanovic 2009). Furthermore, our previous work indicated that retinoic acid (RA) induction of NT2/D1 cells is accompanied by up-regulation of *SOX3* gene expression (Mojsin *et al.* 2006, Stevanovic 2003). This review is focused on the RA-induced regulation of *SOX3* gene expression, with particular emphasis on the involvement of retinoid receptors in this process.

NT2/D1 cell line as a model system for neural differentiation

EC cells are stem cells derived from teratocarcinomas, which arise from transformed germ cells, and are generally considered to be the malignant counterparts of human embryonic stem (ES) cells (Andrews 2002). To date, the most widely characterized EC cell line is NT2/D1. These cells are derived by cloning the NTERA-2 cells established from a nude mouse xenograft of the TERA-2 cell line, which was originally derived from a metastasis of a human testicular teratocarcinoma (Andrews 1984). NT2/D1 cells resemble early ES cells in morphology, antigen expression patterns, biochemistry, developmental potential, and gene regulation (Andrews 1984). Upon culture in RA, NT2/D1 cells differentiate into well developed, morphologic, and immunophenotypic central nervous system-like neurons, with associated loss of cell growth and tumorigenicity (Andrews 1984, Kurie *et al.* 1993, Spinella *et al.* 1999). These cells have properties similar to those of progenitor cells in the central nervous system (CNS), providing an excellent *in vitro* model system for studying human genes that promote and regulate neural differentiation (Spinella *et al.* 2003).

The role of RA in neurodevelopment

Retinoid signaling contributes to development of the CNS (Maden 2002). In lower vertebrates, it is required for generating the adequate numbers of primary neurons, and for their correct positioning (Sharpe and Goldstone 2000). In the embryonic vertebrate CNS, RA has a role in patterning both the anteroposterior and dorsoventral axes (Cunningham *et al.* 1994). Its main sites of action are the hindbrain and anterior spinal cord (Glover *et al.* 2006). RA also regulates interneuron and motor neuron development along the dorsoventral axis (Sockanathan and Jessell 1998).

Moreover, important regulatory role of RA within the adult CNS has been described (Christie *et al.*

2008). Namely, it was shown that specific areas of the brain, known to undergo active remodeling of neural connections throughout adulthood, synthesize RA (the basal ganglia, olfactory bulbs, hippocampus and auditory afferents) (Dev *et al.* 1993). Importantly, the high levels of RA and associated proteins in the hippocampus, the dentate gyrus in particular, strongly implied the involvement of these molecules in region-specific neurogenesis (Jacobs *et al.* 2006). It was revealed that RA contributed significantly to neuronal differentiation within the dentate gyrus, being involved at a very early stage throughout this process (Jacobs *et al.* 2006).

Deregulation of RA signaling has been suggested to be an underlying cause of several neurological disorders, such as Alzheimer's and Parkinson's diseases (Christie *et al.* 2008). Accordingly, understanding the mechanisms of RA action in the developing and adult CNS is an important issue, and recently gained additional significance as a therapeutic strategy.

RA signaling

Retinoic acid is naturally occurring, main vitamin A derivative that plays a critical role in the development and homeostasis of all vertebrate tissues, including cell differentiation, proliferation, metabolism and apoptosis (Altucci and Gronemeyer 2001, Ross *et al.* 2000). The retinoid signal is primarily mediated by two families of nuclear retinoid receptors: RA receptors (RARs; NR1B) and retinoid X receptors (RXRs; NR2B), which work as ligand activated transcription factors in a spatio-temporal specific manner (Kastner *et al.* 1997). There are three different RAR and RXR isotypes (α, β and γ) that are encoded by separate genes at distinct chromosomal loci (Chambon 1996). Members of these two retinoid receptor families form stable heterodimers that, in response to RA binding, modulate the transcription of target genes *via cis*-acting RA response elements (REs), RAREs (Mangelsdorf and Evans 1995). The majority of classical RAREs, specifically recognized by RXR/RAR heterodimers, consist of a direct repeat (DR) of two hexameric half-sites with the consensus sequence 5′-PuG(G/T)TCA-3′, most commonly separated by two or five 'spacer' nucleotides (DR-2 or DR-5, respectively) (Bastien and Rochette-Egly 2004, Laudet and Gronemeyer 2002).

In addition, the biological activity of RA is extended by the fact that RXR acts as a homodimer (IJpenberg *et al.* 2004), or as an obligate heterodimeric partner for a various array of other members of the intracellular receptor superfamily, such as thyroid hormone receptors (TRs), vitamin D receptors (VDRs), peroxisome proliferator-activated receptor (PPAR), farnesoid X-activated receptor (FXR), liver-X receptor (LXR) and other (Laudet and Gronemeyer 2002, Lefebvre *et al.* 2010). These various receptors strongly interact with RXR, and their transcriptional activities are exerted mainly by the resulting heterodimers. RXR thus functions as a "master regulator" of multiple signaling pathways that are essential for mammalian physiology and development (Germain *et al.* 2006). RXR homodimers bind to REs in which the half-sites are separated by 1 bp (DR-1), and RXR containing heterodimers exhibit distinct preferences for a certain spacer length that could be one to five nucleotides (DR-1 to DR-5, respectively) (Laudet and Gronemeyer 2002). Therefore, configuration of these REs, i.e. the arrangement and the spacing between the half-sites, is an important determinant that confers the selectivity and binding specificity of RXR homo/heterodimers (Glass 1994, Laudet and Gronemeyer 2002).

Hundreds of genes have now been shown to be regulated by RA during the processes of neuronal differentiation and neurite outgrowth (Maden 2001). However, only for a minority of them it has been unquestionably shown that are direct targets of the classical RAR–RXR–RARE pathway (Balmer and Blomhoff 2002, Blomhoff and Blomhoff 2006). In many cases, the gene regulation appears to be indirect, reflecting the actions of intermediate transcription factors, non-classical associations of receptors with other proteins, or even more distant mechanisms (Blomhoff and Blomhoff 2006).

Many RA-regulated genes have been discovered in human EC cells. However, precise understanding of the particular gene regulation by retinoids in these cells is yet to be accomplished (Soprano *et al.* 2007). Therefore, the study of the human *SOX3* gene expression, for which we have shown that is a direct RA downstream target in NT2/D1 EC model system, is valuable for future investigation of molecular events underlying EC cells neuronal differentiation following RA treatment.

Localization of RA responsive regions and involvement of retinoid receptors in the regulation of *SOX3* gene expression

Initially, we showed that *SOX3* gene is expressed in NT2/D1 cells and that early phases of differentiation and

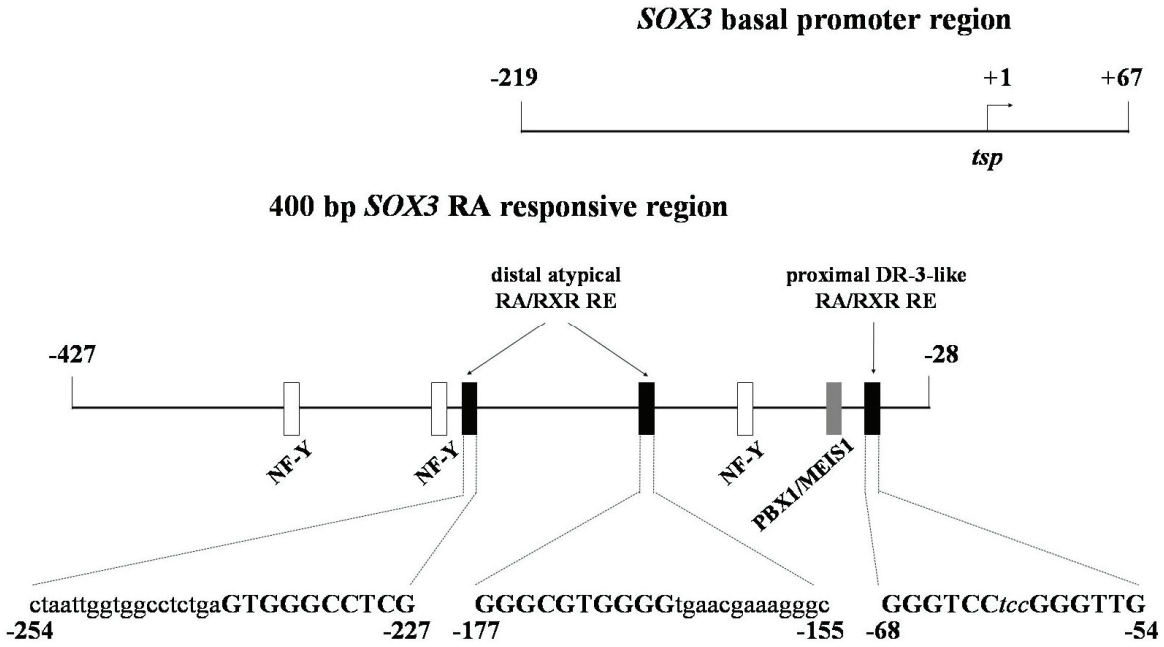

Fig. 1. Schematic presentation of the human *SOX3* promoter region, with delineated RXR *cis*-regulatory elements. Two positive regulatory promoter elements involved in RA/RXR-dependent activation of the human *SOX3* gene expression in NT2/D1 cells were identified: distal RE that consists of two unique G-rich boxes separated by 49 bp, which could be considered as an atypical RA-response element, and proximal RE that represents a DR-3-like motif, composed of two imperfect hexameric DRs, and is characterized as functional RE. Distal atypical RA/RXR RE: capital letters in bold represent regions that are necessary and sufficient for RXRα binding, lowercase letters mark the extended regions needed for higher binding affinity. Proximal DR-3-like RXR RE: two hexameric halfsites are represented by capital letters in bold, with 3 bp spacer indicated by italic lowercase letters. The binding sites for transcription factors (NF-Y and PBX1/MEIS1) also demonstrated to be involved in the RA-induced up-regulation of *SOX3* gene expression are indicated. All positions are given relative to *tsp.*

neural induction, which take place within 48 h of RA exposure, involve up-regulation of this gene expression at both mRNA and protein levels (Mojsin *et al.* 2006, Stevanovic 2003). This is also in correlation with the literature data based on microarray analysis (Freemantle *et al.* 2002). Since the molecular mechanism(s) underlying regulation of *SOX3* gene by RA are still unknown, our first goal was to search for regions within *SOX3* promoter that mediate RA-induced transcription.

Using enhancer-dependent reporter plasmid approach, we showed that regulatory elements responsible for both, basal and RA-induced transcriptional activation of *SOX3* gene are localized within the 400 bp of its 5'-flanking region (Mojsin *et al.* 2006). To explore whether the *SOX3* induction is mediated by retinoid receptors, overexpression studies with RXRα were employed. The RXRα has been chosen as the representative of retinoid receptors, to serve as a tool in the study of *SOX3* gene responsiveness to RA. Overexpression of RXRα resulted in up-regulation of *SOX3* gene expression in response to RA (Mojsin *et al.* 2006). These results confirmed that RA effect on *SOX3* gene expression is mediated by retinoid receptors.

Localization of RA/RXR response elements

A more systematic search for regulatory regions that are able to mediate responsiveness to RA was performed by deletion mapping of the *SOX3* 400 bp 5'-flanking region (Mojsin *et al.* 2006, Nikcevic *et al.* 2008). Two response elements involved in RA/RXR-dependent activation of the *SOX3* gene expression were identified (Mojsin *et al.* 2006, Nikcevic *et al.* 2008), designated as distal and proximal REs (Fig. 1).

The distal RA/RXR response element is pinned down to two regulatory elements (Mojsin *et al.* 2006). Only in the presence of both elements, full RA/RXR inducibility is achieved, suggesting they act synergistically. By DNase I footprinting and gel shift analyses, RXR binding motifs were determined, revealing that these regulatory elements comprise two unique G-rich boxes separated by 49 bp (Mojsin *et al.* 2006). Since these motifs did not show any homology to known nuclear receptor REs commonly present in RA-responsive genes, we have proposed it should be considered as a novel, atypical RA/RXR response element (Mojsin *et al.* 2006) (Fig. 1). Interestingly,

literature data indicate that RAREs can be increasingly diverse in the core consensus motifs, with unusual spacing or symmetry attributes (Balmer and Blomhoff 2005). For example, activation of the Burkitt lymphoma receptor 1 gene (*blr1*) by RA seems to depend on RARE (17 bp in length), that contains two, arbitrarily named, GT box elements (Wang and Yen 2004). Also, composite response unit that encompasses two degenerate, GC-rich sequences, separated by 30 nucleotides, mediates RA induction of the human retinol-binding protein gene (*RBP*) (Panariello *et al.* 1996).

Further deletion analysis pointed to the presence of an additional, proximal *SOX3* regulatory region responsive to RA that contains an RA/RXR responsive region, as determined by overexpression of RXRα in NT2/D1 (Nikcevic *et al.* 2006, Nikcevic *et al.* 2008). By competition and scanning gel-shift analyses, this region was narrowed down to a 31 bp fragment that directly interacts with recombinant RXRα. Furthermore, by gel-shift mutation analysis of 31 bp fragment, we identified the sequence 5′-**GGGTCC***tcc***GGGTTG-**3′ as an RXR binding site (Nikcevic *et al.* 2008). It represents a DR-3-like motif, composed of two hexameric half-sites, separated by 3 bp (Fig. 1).

In addition, functional significance of the DR-3-like element was indicated by mutational analyses, which showed that disruption/mutation of this element caused significant reduction of RA/RXR transactivation of the *SOX3* promoter (Nikcevic *et al.* 2008). Moreover, the crucial evidence for functional relevance of this regulatory region came from results showing that 31 bp oligonucleotide, encompassing the DR-3-like element, is capable of independently mediating the RA/RXR effect in a heterologous promoter context (Nikcevic *et al.* 2008).

Involvement of retinoid receptors in the regulation of *SOX3* gene expression

Testing the binding ability of the described DR-3-like motif revealed that both recombinant and RXRα from nuclear extracts of RA-treated NT2/D1 cells interact with this motif in a sequence-specific manner. In addition, we have analyzed the potential binding of RARs and VDRs to the DR-3-like *SOX3* RE. The involvement of nuclear receptor VDR was analyzed because the identified DR-3 binding site resembled the configuration of RE characteristic for vitamin D receptors (Aranda and Pascual 2001). However, our experiments suggested that neither RARs nor VDRs participate in complex formation as RXRs heterodimeric partners (Nikcevic *et al.* 2008). This finding emphasized the need to continue study of the *SOX3* gene responsiveness to RA in natural settings, and for that purpose, we have employed synthetic retinoids.

Specifically, advances in the understanding of nuclear receptors at both structural and functional level enabled the design of ligands, selective receptor modulators that have specific agonist or antagonist features (Altucci *et al.* 2007, de Lera *et al.* 2007). In order to assess the involvement of each class of retinoid receptors in RA-induced SOX3 up-regulation on the endogenous level, we treated NT2/D1 cells with RXRs and RARs pan-antagonists (LG101208 and LG100815, respectively) in the presence of RA (Nikcevic *et al.* 2008). The results of western blot analysis showed that the RA induction of SOX3 protein expression was reduced in the presence of RXR antagonist, whereas the treatment with RAR antagonist did not markedly alter the RA effect on SOX3 expression (Nikcevic *et al.* 2008). This data pointed to RXRs, but not RARs, as mediators of the RA effect on the endogenous SOX3 up-regulation in NT2/D1 cells. It is important to underline that by using this approach, we showed for the first time, that RA-induced *SOX3* gene expression could be significantly down-regulated by the synthetic antagonist of RXR.

The observed RA effect on *SOX3* gene expression that relies on RXR and not on RAR, might indicate integration of RA and another signaling pathway(s) through defined RXR binding site(s) within the *SOX3* promoter. In that view, potential RXR partner(s), whose activity might be ligand dependent, would be particularly interesting implying another signaling that together with RA pathway could be responsible for the fine-tuning of *SOX3* gene regulation.

We also studied potential involvement of RXR homodimers in mediating the RA effect on the up-regulation of *SOX3* gene expression in NT2/D1 cells. For that purpose, we used a potent and efficacious activator of RXR homodimers (LG100268). Results of western blot analysis showed that RXR homodimers are not responsible for mediating the RA effect on endogenous SOX3 gene expression (Savic *et al.* 2009).

Additional experiments, focusing on defining the RXR partners, are necessary for the precise characterization of this RA–RXR–SOX3 signaling and its wider biological significance.

The involvement of other transcription factors in the up-regulation of *SOX3* gene expression by RA

Our work pointed to multiple CCAAT box control elements within the *SOX3* promoter that could be recognized as modulators of RA-induced activation of *SOX3* gene expression (Krstic *et al.* 2007). Also, we recently showed that the TALE (three-amino-acid loop extension) transcription factors PBX1 and MEIS1 participate in regulating RA-dependent up-regulation of *SOX3* gene expression. It appears that PBX1/MEIS1 directly interact with the binding site within *SOX3* basal promoter region, which is conserved in all analyzed mammalian orthologues, and that these transcription factors are responsible, at least in part, for *SOX3* responsiveness to RA (Mojsin and Stevanovic 2010).

It is also interesting that NF-Y and TR/RXR nuclear receptors functionally interact to confer triiodothyronine (T3)-stimulated transactivation of the hepatic S14 gene (Jump *et al.* 1997). Furthermore, it was reported that PBX1/MEIS1 heterodimers interact with TR/RXR complex to enhance T3 regulation of malic enzyme transcription in hepatocytes (Wang *et al.* 2001).

In accordance with these data, it is reasonable to speculate that accurate expression of the *SOX3* gene during specific stages of development depends on differential usage and/or interplay of the described multiple RAREs, PBX1/MEIS1 and NF-Y binding sites within the promoter of this gene (Fig. 1). Accordingly, further studies are needed to identify the coordinated action of nuclear receptors, NF-Y, PBX1/MEIS1, and other, not yet identified transcription factor(s), in the up-regulation of *SOX3* gene expression during early stages of RA-induced neural differentiation of NT2/D1 cells.

Significance and future directions

Recently, considerable attention has been focused on understanding the molecular basis of pluripotency and the earliest differentiation processes. The knowledge gained through these studies would pave the path to grow and manipulate pluripotent ES cells efficiently, reproducibly and in a manner appropriate for clinical applications (Johnson *et al.* 2008). NT2/D1 cells are considered as an important alternative to stem cells, having properties similar to those of progenitor cells in the CNS. Therefore, elucidating mechanism(s) underlying regulation of expression of *SOX3* gene in this human EC cells model system will improve the understanding of molecular signals that induce neurogenesis in the stem/progenitor cells both during development and in adulthood. The presented line of study should also accelerate the evaluation of *SOX* genes as potential targets for modulation of proliferation and differentiation of neural progenitors.

Because the neuronal loss is a common feature of many neurological disorders, including stroke, Parkinson's and Alzheimer's diseases, and traumatic brain injury, it is worthy highlighting the following. First, after exposure to RA, NT2 cells were used for transplantation as cell therapy for brain injury, ischemia, and neurodegenerative diseases in animal models as well as in two clinical trials of human stroke patients (Newman *et al.* 2005). Second, it was postulated that RA-dependent molecular cascade could play a central role in the intrinsic regenerative capacity of the CNS (Malaspina and Michael-Titus 2008). However, despite the recent progress, the replacement of lost cells, either by cell transplantation or by the manipulation of patient's progenitor cells *in situ*, is still far from the routine therapeutic practice. In that respect, it is essential to dissect each step of adult neurogenesis in order to enable selection of those mechanisms that could be targets for potential pharmaceutical approaches and future clinical applications. We believe that elucidating mechanism(s) underlying regulation of expression of human *SOX3* gene in NT2/D1 cells could represent valuable contribution to this field.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The research on human *SOX3* gene has been supported by the Ministry of Science and Technological Development, Republic of Serbia (Grant numbers 143028 and 173051).

References

ALTUCCI L, GRONEMEYER H: Nuclear receptors in cell life and death. *Trends Endocrinol Metab* **12**: 460-468, 2001.

ALTUCCI L, LEIBOWITZ MD, OGILVIE KM, DE LERA AR, GRONEMEYER H: RAR and RXR modulation in cancer and metabolic disease. *Nat Rev Drug Discov* **6**: 793-810, 2007.

ANDREWS PW: From teratocarcinomas to embryonic stem cells. *Philos Trans R Soc Lond B Biol Sci* **357**: 405-417, 2002.

ANDREWS PW: Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. *Dev Biol* **103**: 285-293, 1984.

ARANDA A, PASCUAL A: Nuclear hormone receptors and gene expression. *Physiol Rev* **81**: 1269-1304, 2001.

BALMER JE, BLOMHOFF R: Gene expression regulation by retinoic acid. *J Lipid Res* **43**: 1773-1808, 2002.

BALMER JE, BLOMHOFF R: A robust characterization of retinoic acid response elements based on a comparison of sites in three species. *J Steroid Biochem Mol Biol* **96**: 347-354, 2005.

BASTIEN J, ROCHETTE-EGLY C: Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* **328**: 1-16, 2004.

BLOMHOFF R, BLOMHOFF HK: Overview of retinoid metabolism and function. *J Neurobiol* **66**: 606-630, 2006.

BOWLES J, SCHEPERS G, KOOPMAN P: Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev Biol* **227**: 239-255, 2000.

BRUNELLI S, SILVA CASEY E, BELL D, HARLAND R, LOVELL-BADGE R: Expression of Sox3 throughout the developing central nervous system is dependent on the combined action of discrete, evolutionarily conserved regulatory elements. *Genesis* **36**: 12-24, 2003.

BYLUND M, ANDERSSON E, NOVITCH BG, MUHR J: Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat Neurosci* **6**: 1162-1168, 2003.

CHAMBON P: A decade of molecular biology of retinoic acid receptors. *FASEB J* **10**: 940-954, 1996.

CHRISTIE VB, MARDER TB, WHITING A, PRZYBORSKI SA: The role of retinoids in the adult nervous system and their therapeutic potential. *Mini Rev Med Chem* **8**: 601-608, 2008.

CUNNINGHAM ML, MAC AULEY A, MIRKES PE: From gastrulation to neurulation: transition in retinoic acid sensitivity identifies distinct stages of neural patterning in the rat. *Dev Dyn* **200**: 227-241, 1994.

DE LERA AR, BOURGUET W, ALTUCCI L, GRONEMEYER H: Design of selective nuclear receptor modulators: RAR and RXR as a case study. *Nat Rev Drug Discov* **6**: 811-820, 2007.

DEV S, ADLER AJ, EDWARDS RB: Adult rabbit brain synthesizes retinoic acid. *Brain Res* **632**: 325-328, 1993.

EKONOMOU A, KAZANIS I, MALAS S, WOOD H, ALIFRAGIS P, DENAXA M, KARAGOGEOS D, CONSTANTI A, LOVELL-BADGE R, EPISKOPOU V: Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1. *PLoS Biol* **3**: e186, 2005.

FERRI AL, CAVALLARO M, BRAIDA D, DI CRISTOFANO A, CANTA A, VEZZANI A, OTTOLENGHI S, PANDOLFI PP, SALA M, DEBIASI S, NICOLIS SK: Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development* **131**: 3805-3819, 2004.

FREEMANTLE SJ, KERLEY JS, OLSEN SL, GROSS RH, SPINELLA MJ: Developmentally-related candidate retinoic acid target genes regulated early during neuronal differentiation of human embryonal carcinoma. *Oncogene* **21**: 2880-2889, 2002.

GERMAIN P, CHAMBON P, EICHELE G, EVANS RM, LAZAR MA, LEID M, DE LERA AR, LOTAN R, MANGELSDORF DJ, GRONEMEYER H: International Union of Pharmacology. LXIII. Retinoid X receptors. *Pharmacol Rev* **58**: 760-772, 2006.

GLASS CK: Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr Rev* **15**: 391-407, 1994.

GLOVER JC, RENAUD JS, RIJLI FM: Retinoic acid and hindbrain patterning. *J Neurobiol* **66**: 705-725, 2006.

IJPENBERG A, TAN NS, GELMAN L, KERSTEN S, SEYDOUX J, XU J, METZGER D, CANAPLE L, CHAMBON P, WAHLI W, DESVERGNE B: In vivo activation of PPAR target genes by RXR homodimers. *Embo J* **23**: 2083-2091, 2004.

JACOBS S, LIE DC, DECICCO KL, SHI Y, DELUCA LM, GAGE FH, EVANS RM: Retinoic acid is required early during adult neurogenesis in the dentate gyrus. *Proc Natl Acad Sci U S A* **103**: 3902-3907, 2006.

JOHNSON BV, SHINDO N, RATHJEN PD, RATHJEN J, KEOUGH RA: Understanding pluripotency – how embryonic stem cells keep their options open. *Mol Hum Reprod* **14**: 513-520, 2008.

JUMP DB, BADIN MV, THELEN A: The CCAAT box binding factor, NF-Y, is required for thyroid hormone regulation of rat liver S14 gene transcription. *J Biol Chem* **272**: 27778-27786, 1997.

KAMACHI Y, UCHIKAWA M, KONDOH H: Pairing SOX off: with partners in the regulation of embryonic development. *Trends Genet* **16**: 182-187, 2000.

KASTNER P, MARK M, GHYSELINCK N, KREZEL W, DUPE V, GRONDONA JM, CHAMBON P: Genetic evidence that the retinoid signal is transduced by heterodimeric RXR/RAR functional units during mouse development. *Development* **124**: 313-326, 1997.

KOVACEVIC GRUJICIC N, MOJSIN M, KRSTIC A, STEVANOVIC M: Functional characterization of the human SOX3 promoter: identification of transcription factors implicated in basal promoter activity. *Gene* **344**: 287-297, 2005.

KOVACEVIC GRUJICIC N, YOKOYAMA K, STEVANOVIC M: Trans-activation of the human SOX3 promoter by MAZ in NT2/D1 cells. *Arch Biol Sci* **60**: 379-387, 2008.

KRSTIC A, MOJSIN M, STEVANOVIC M: Regulation of SOX3 gene expression is driven by multiple NF-Y binding elements. *Arch Biochem Biophys* **467**: 163-173, 2007.

KURIE JM, BUCK J, EPPINGER TM, MOY D, DMITROVSKY E: 9-cis and all-trans retinoic acid induce a similar phenotype in human teratocarcinoma cells. *Differentiation* **54**: 123-129, 1993.

LAUDET V, GRONEMEYER H: *The Nuclear Receptor Factsbook*. Academic Press, San Diego, 2002.

LAUMONNIER F, RONCE N, HAMEL BC, THOMAS P, LESPINASSE J, RAYNAUD M, PARINGAUX C, VAN BOKHOVEN H, KALSCHEUER V, FRYNS JP, CHELLY J, MORAINE C, BRIAULT S: Transcription factor SOX3 is involved in X-linked mental retardation with growth hormone deficiency. *Am J Hum Genet* **71**: 1450-1455, 2002.

LEFEBVRE P, BENOMAR Y, STAELS B: Retinoid X receptors: common heterodimerization partners with distinct functions. *Trends Endocrinol Metab* **21**: 676-683, 2010.

LEFEBVRE V, DUMITRIU B, PENZO-MENDEZ A, HAN Y, PALLAVI B: Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. *Int J Biochem Cell Biol* **39**: 2195-2214, 2007.

MADEN M: Retinoid signalling in the development of the central nervous system. *Nat Rev Neurosci* **3**: 843-853, 2002.

MADEN M: Role and distribution of retinoic acid during CNS development. *Int Rev Cytol* **209**: 1-77, 2001.

MALASPINA A, MICHAEL-TITUS AT: Is the modulation of retinoid and retinoid-associated signaling a future therapeutic strategy in neurological trauma and neurodegeneration? *J Neurochem* **104**: 584-595, 2008.

MANGELSDORF DJ, EVANS RM: The RXR heterodimers and orphan receptors. *Cell* **83**: 841-850, 1995.

MOJSIN M, GRUJICIC NK, NIKCEVIC G, KRSTIC A, SAVIC T, STEVANOVIC M: Mapping of the RXRalpha binding elements involved in retinoic acid induced transcriptional activation of the human SOX3 gene. *Neurosci Res* **56**: 409-418, 2006.

MOJSIN M, STEVANOVIC M: PBX1 and MEIS1 up-regulate SOX3 gene expression by direct interaction with a consensus binding site within the basal promoter region. *Biochem J* **425**: 107-116, 2010.

NEWMAN MB, MISIUTA I, WILLING AE, ZIGOVA T, KARL RC, BORLONGAN CV, SANBERG PR: Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells. *Stem Cells Dev* **14**: 29-43, 2005.

NIKCEVIC G, KOVACEVIC GRUJICIC N, STEVANOVIC M: Activation of the HSV-TK promoter in control reporter vector pBLCAT5 by liganded nuclear retinoid receptor RXRalpha. *Arch Biol Sci, Belgrade* **58**: 197-203, 2006.

NIKCEVIC G, SAVIC T, KOVACEVIC-GRUJICIC N, STEVANOVIC M: Up-regulation of the SOX3 gene expression by retinoic acid: characterization of the novel promoter-response element and the retinoid receptors involved. *J Neurochem* **107**: 1206-1215, 2008.

PANARIELLO L, QUADRO L, TREMATERRA S, COLANTUONI V: Identification of a novel retinoic acid response element in the promoter region of the retinol-binding protein gene. *J Biol Chem* **271**: 25524-25532, 1996.

PEVNY L, PLACZEK M: SOX genes and neural progenitor identity. *Curr Opin Neurobiol* **15**: 7-13, 2005.

PEVNY LH, LOVELL-BADGE R: Sox genes find their feet. *Curr Opin Genet Dev* **7**: 338-344, 1997.

RIZZOTI K, BRUNELLI S, CARMIGNAC D, THOMAS PQ, ROBINSON IC, LOVELL-BADGE R: SOX3 is required during the formation of the hypothalamo-pituitary axis. *Nat Genet* **36**: 247-255, 2004.

ROGERS CD, ARCHER TC, CUNNINGHAM DD, GRAMMER TC, CASEY EM: Sox3 expression is maintained by FGF signaling and restricted to the neural plate by Vent proteins in the Xenopus embryo. *Dev Biol* **313**: 307-319, 2008.

ROSS SA, MCCAFFERY PJ, DRAGER UC, DE LUCA LM: Retinoids in embryonal development. *Physiol Rev* **80**: 1021-1054, 2000.

SAVIC T, STEVANOVIC M, NIKCEVIC G: Retinoic acid-induced SOX3 gene expression in NT2/D1 cells is RXR homodimer-independent. *Arch Biol Sci, Belgrade* **61**: 631-638, 2009.

SHARPE C, GOLDSTONE K: The control of Xenopus embryonic primary neurogenesis is mediated by retinoid signalling in the neurectoderm. *Mech Dev* **91**: 69-80, 2000.

SOCKANATHAN S, JESSELL TM: Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. *Cell* **94**: 503-514, 1998.

SOPRANO DR, TEETS BW, SOPRANO KJ: Role of retinoic Acid in the differentiation of embryonal carcinoma and embryonic stem cells. *Vitam Horm* **75**: 69-95, 2007.

SPINELLA MJ, FREEMANTLE SJ, SEKULA D, CHANG JH, CHRISTIE AJ, DMITROVSKY E: Retinoic acid promotes ubiquitination and proteolysis of cyclin D1 during induced tumor cell differentiation. *J Biol Chem* **274**: 22013-22018, 1999.

SPINELLA MJ, KERLEY JS, WHITE KA, CURTIN JC: Retinoid target gene activation during induced tumor cell differentiation: human embryonal carcinoma as a model. *J Nutr* **133**: 273S-276S, 2003.

STANKIEWICZ P, THIELE H, SCHLICKER M, CSEKE-FRIEDRICH A, BARTEL-FRIEDRICH S, YATSENKO SA, LUPSKI JR, HANSMANN I: Duplication of Xq26.2-q27.1, including SOX3, in a mother and daughter with short stature and dyslalia. *Am J Med Genet A* **138**: 11-17, 2005.

STEVANOVIC M: Modulation of SOX2 and SOX3 gene expression during differentiation of human neuronal precursor cell line NTERA2. *Mol Biol Rep* **30**: 127-132, 2003.

STEVANOVIC M: Transcriptional regulation of the human SOX3 gene expression: Facts from the human embryonal carcinoma cells. In: *Recent Research Developments in Neuroscience*. PANDALAI SG (ed), Research Signpost, Kerala, 2009, pp 79-98.

STEVANOVIC M, LOVELL-BADGE R, COLLIGNON J, GOODFELLOW PN: SOX3 is an X-linked gene related to SRY. *Hum Mol Genet* **2**: 2013-2018, 1993.

UCHIKAWA M, KAMACHI Y, KONDOH H: Two distinct subgroups of Group B Sox genes for transcriptional activators and repressors: their expression during embryonic organogenesis of the chicken. *Mech Dev* **84**: 103-120, 1999.

WANG J, YEN A: A novel retinoic acid-responsive element regulates retinoic acid-induced BLR1 expression. *Mol Cell Biol* **24**: 2423-2443, 2004.

WANG Y, YIN L, HILLGARTNER FB: The homeodomain proteins PBX and MEIS1 are accessory factors that enhance thyroid hormone regulation of the malic enzyme gene in hepatocytes. *J Biol Chem* **276**: 23838-23848, 2001.

WEGNER M: From head to toes: the multiple facets of Sox proteins. *Nucleic Acids Res* **27**: 1409-1420, 1999.

WEISS J, MEEKS JJ, HURLEY L, RAVEROT G, FRASSETTO A, JAMESON JL: Sox3 is required for gonadal function, but not sex determination, in males and females. *Mol Cell Biol* **23**: 8084-8091, 2003.

WILSON M, KOOPMAN P: Matching SOX: partner proteins and co-factors of the SOX family of transcriptional regulators. *Curr Opin Genet Dev* **12**: 441-446, 2002.

WOODS KS, CUNDALL M, TURTON J, RIZOTTI K, MEHTA A, PALMER R, WONG J, CHONG WK, AL-ZYOUD M, EL-ALI M, OTONKOSKI T, MARTINEZ-BARBERA JP, THOMAS PQ, ROBINSON IC, LOVELL-BADGE R, WOODWARD KJ, DATTANI MT: Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet* **76**: 833-849, 2005.