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INVOLVEMENT OF UBIQUITOUS AND TALE TRANSCRIPTION FACTORS, AS WELL AS LIGANDED RXRα, IN THE REGULATION OF HUMAN SOX2 GENE EXPRESSION IN THE NT2/D1 EMBRYONAL CARCINOMA CELL LINE

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Abstract - SOX2 is a key transcription factor in embryonic development representing a universal marker of pluripotent stem cells. Based on the functional redundancy and overlapping expression patterns of SOXB1 subgroup members during development, the goal of this study has been to analyze if some aspects of regulation of expression are preserved between human SOX2 and SOX3 genes. Thus, we have tested several transcription factors previously demonstrated to play roles in controlling SOX3 gene activity for potential participation in the regulation of SOX2 gene expression in NT2/D1 cells. Here we report on the activation of SOX2 expression by ubiquitous transcription factors (NF-Y, Sp1 and MAZ), TALE family members (Pbx1 and Meis1), as well as liganded RXRα. Elucidating components involved in the regulation of SOX gene expression represent a valuable contribution in unraveling the regulatory networks operating in pluripotent embryonic cells.

Key words: Human SOX2, NT2/D1 embryonal carcinoma cell line, NF-Y, Sp1, MAZ, PBX1, MEIS1, RXRα.

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INTRODUCTION

The *SOX* gene family encodes a highly conserved group of transcription factors that control cell fate and differentiation in numerous key developmental and physiological processes (reviewed in Lefebvre et al., 2007). SOX transcription factors carry an DNA-binding HMG domain and perform their functions by interacting 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, either activating or repressing specific target genes (Wilson and Koopman, 2002). Based on phylogenetic analysis of their HMG domains, *Sox* genes are divided into 10 distinct groups designated A-J (Bowles et al., 2000).

SOX2, together with SOX1 and SOX3, belongs to the SOXB1 subgroup of transcriptional activators (Uchikawa et al., 1999). They are panneurally expressed and have redundant roles in maintaining the broad developmental potential and identity of neural stem cells (reviewed in Lefebvre et al., 2007). Their inhibition in the vertebrate embryo results in premature differentiation of neural precursors, while their overexpression leads to inhibition of neurogenesis (reviewed in Lefebvre et al., 2007). In addition to regulating the progression of neurogenesis, SOXB1 transcription factors (TFs) also function in post-mitotic neurons (Kiefer, 2007).

Sox2 is a key TF in embryonic development and represents an universal marker of pluripotent stem cells (Papanayotou et al., 2008). Numerous studies recognize Sox2, together with Nanog and Oct3/4 transcription factors, as crucial for maintaining the pluripotent stem cell phenotype (Rodda et al., 2005). It has recently been shown that Sox2, together with Oct3/4, c-Myc and Klf4, forms a quartet of transcription factors that is sufficient to induce pluripotent stem cell properties in embryo-

nic and adult fibroblasts (Takahashi and Yamanaka, 2006). Thus, it has been proposed that Sox2 probably functions as a molecular rheostat that controls the expression of a critical set of embryonic genes, as well as the self-renewal and differentiation of embryonic stem (ES) cells (Kopp et al., 2008).

Although *Sox2* is expressed panneurally, its transcriptional regulation in the CNS is differentially regulated by regional signals, according to the specific developmental needs of the different regions of the nervous system (Catena et al., 2004). Therefore, it is not surprising that the complex expression profile of *Sox2* is controlled by multiple regulatory elements, widely scattered in a genomic region centered around the gene and with distinct spatio-temporal specificities (reviewed in Kamachi et al., 2009). Namely, eleven functionally defined enhancers have been described within 50-kb of the chicken genomic region encompassing the *Sox2* gene (~ 17-kb upstream and ~ 33-kb downstream of the *Sox2* gene). DNA sequences of these enhancers are conserved in a wide range of vertebrate species, where they are embedded in much longer stretches of highly conserved, non-functional sequences surrounding the *Sox2* gene (reviewed in Kamachi et al., 2009).

Apart from these far-distance *Sox2* enhancers' studies, functional analyses of the mouse *Sox2* promoter have been performed as well. Namely, it has been shown that the regulatory region (-528 to +238) of the *Sox2* gene is able to support the expression of the reporter gene in mouse embryonal carcinoma (EC) cells, preferentially when they are in an undifferentiated state (Wiebe et al., 2000). Moreover, it has been found that the same regulatory region retains its activity when the EC and ES cells were induced to differentiate (Wiebe et al., 2000). It has also been revealed that the murine *Sox2* promoter region, located -426 to +9, is able to regulate the expression of this gene via the agonistic and antagonistic action of AP2, Msx2, Pax6, Prox1 and Six3 transcription factors (Lengler et al., 2005).

To date, the most widely characterized pluripotential EC cell line is the human NT2/D1 cell line that resembles early ES cells in morphology, antigen expression patterns, biochemistry, developmental potential, and gene regulation (Andrews, 1984). Upon culture in retinoic acid (RA), NT2/D1 cells differentiate into well-developed CNS-like neurons (Spinella et al., 2003). Accordingly, these cells serve as a powerful *in vitro* model system for studying genes that promote and regulate human neural differentiation (Spinella et al., 2003).

We have previously described the modulation of *SOX2* and *SOX3* gene expression during the early phases of differentiation and neural induction of NT2/D1 cells by RA (Stevanovic, 2003). Also, we have performed extensive functional characterization of the human *SOX3* promoter (Kovacevic Grujicic et al., 2005) and demonstrated the involvement of several transcription factors (TFs) in the regulation of expression of this gene (Kovacevic Grujicic et al., 2005; Krstic et al., 2007; Mojsin et al., 2006; Nikcevic et al., 2008).

Based on the functional redundancy and overlapping expression patterns of SOXB1 members during development, the goal of the present study has been to analyze whether some aspects of regulation of expression are preserved between human *SOX2* and *SOX3* genes.

MATERIALS AND METHODS

Cell culture

NT2/D1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, high glucose) supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine at 37 °C in 10% CO_2. All indicated reagents were obtained from Invitrogen.

Transfection

In transfection experiments 2 x 10^6 NT2/D1 cells were seeded into a 10 cm dish and transfected with 5 μg of expression vectors (NF-YA, pCMVSp1, pCMV-MAZ, pCIPbx1b, pCIMeis1a and pCDNA3.1TGIF), or 1 μg of expression plasmid for nuclear receptor, pRS-hRXRα. The vectors pCIPbx1b and pCIMeis1a were

generated by releasing mouse Pbx1b and Meis1a cDNAs from pGEM3zf-Pbx1b and MSCV-Meis1a, respectively (kind gift from Prof. Mark P. Kamps), and cloning in a pCI mammalian expression vector (Promega). Vector pBluescript (Stratagene) was used to adjust the total amount of DNA (up to 9 μg for RXRα, and up to 10 μg for all other expression vectors). Cells were transfected using the calcium phosphate precipitation method, as described (Nikcevic et al., 2006).

Western blot analysis

Whole cell lysates (WCL) were prepared from either NT2/D1 cells alone, or transfected with expression vectors for particular transcription factors. In experiments where the nuclear receptor RXRα was involved, the day after transfection the cells were induced by 10μM all-trans RA (Sigma) for a period of 48 h. For each WCL approximately 10^7 cells were scraped and twice washed in 1x phosphate-buffered saline. Cells were lysed for 30 min in 1 mL of cold lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40) containing Protease inhibitor cocktail (Roche). Cell lysates were centrifuged at 10 000 x g for 15 min at 4°C. Supernatants were collected and stored at -80°C. WCL samples were separated by SDS–PAGE in 10% resolving gel using Bio-Rad minigel apparatus and then electrotransferred to PVDF Immobilon P (Millipore). After blocking with 10% nonfat milk at room temperature for 1 h, the membranes were incubated with primary antibodies against SOX2 (ab15830 - Abcam) and anti-α-tubulin (CP06-CALBIOCHEM) under the same conditions. The membranes were then incubated with HRP-conjugated anti-rabbit IgG or anti-mouse IgG (Amersham Pharmacia Biotech) for 1 h at room temperature. Immunoreactive bands were detected using an ECL kit (GE Healtcare). Antibodies for SOX2 and α-tubulin recognize bands of 42 and 55 kDa, respectively.

Immunoblots were digitalized and quantified with the ImageJ program (www.rsb.info.nih.gov/ij). α-tubulin was employed to indicate the amounts of proteins used for the analysis. The quantities of the SOX2 protein level were calculated relative to the non-transfected NT2/D1 cells (set as 1), and were indicated below the particular immunoreactive bands.

Bioinformatic analysis

Database searches were performed using the National Center for Biotechnology Information (NCBI).

To analyze the SOX2 promoter region, the human genome sequence NT_005612.16 retrieved from NCBI was subjected to promoter prediction software PromoterInspector (http://www.genomatix.de).

By applying ClustalW software (EMBL-EBI) and GeneDoc software version 2.6.003 (Nicholas Karl and Nicholas H. B. Jr 1997 Gene Doc: a tool for editing and annotating multiple sequence alignments. Distributed by authors), we performed an alignment of the predicted human SOX2 promoter sequence with the mouse orthologue (retrieved from genome sequence NW_001030719.1).

Transcription factor binding sites within the sequences of interest were analyzed using MatInspector software (Genomatix, http://www.genomatix.de). All predicted transcription factor binding sites have core similarity 1.

RESULTS AND DISCUSSION

The study presented here was undertaken in order to examine the effect of selected transcription factors, which we have previously demonstrated to play roles in controlling *SOX3* gene activity, for their potential participation in the regulation of SOX2 expression in a native context. Accordingly, we analyzed the effect of overexpression of the following TFs: ubiquitous (NF-Y, Sp1, MAZ), TALE (PBX, MEIS, TGIF) and nuclear receptors (retinoid X receptor) on the SOX2 expression in NT2/D1 cells by Western blot analysis. Therefore, the goal of the present study has been to analyze whether some aspects of regulation of expression are preserved between human *SOX2* and *SOX3* genes.

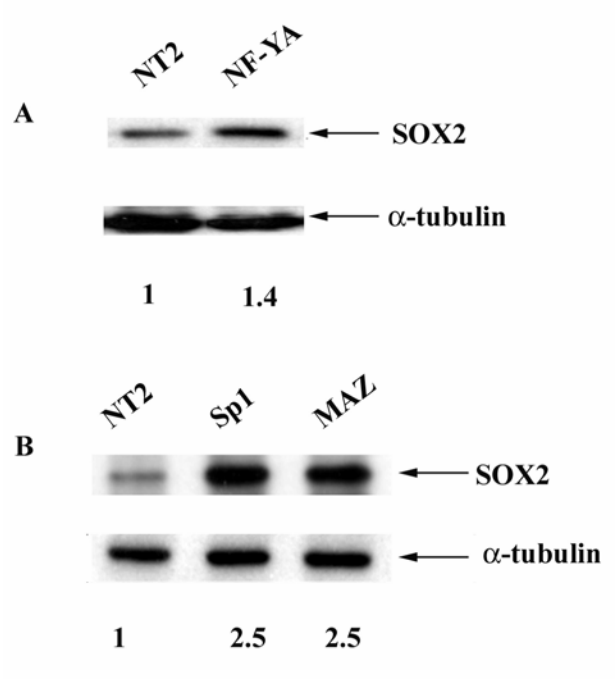

Fig. 1. (A) and (B). Ubiquitous TFs: NF-YA, Sp1 and MAZ up-regulate human SOX2 protein expression. Western blot analyses of WCLs from NT2/D1 cells, non-transfected, or transfected with expression vectors for indicated TFs were performed using antibodies specific for SOX2 and α-tubulin, as indicated on the right. Quantitation of SOX2 protein level (relative to non-transfected NT2/D1 cells which was set as 1) is shown below.

Effect of ubiquitous transcription factors on SOX2 expression

Effects of transcription factors NF-Y and Sp1

The CCAAT box is a widespread eukaryotic promoter element, recognized by a number of TFs such as c/EBP, MS-Y, CTF/NF-1 and NF-Y (Mantovani 1998; Suzuki et al., 2001). Among them, NF-Y is the only one that requires the CCAAT pentanucleotide sequence (Dorn et al., 1987). NF-Y is composed of three subunits (A, B and C), all necessary for DNA binding (Sinha et al., 1995). Upon binding to DNA, NF-Y is able to bend the DNA, indicating that this factor may have an important architectural role (Ronchi et al., 1995). Generally, NF-Y promotes and/or stabilizes the binding of TFs to nearby DNA-binding elements, attracts co-activators and, consequently, enhances transcription (Frontini et al., 2002; Ronchi et al., 1995).

Recently, we have shown that the human *SOX3* promoter possesses three CCAAT box control elements and that all of them mediate transcriptional activation of the *SOX3* gene by binding the transcription factor NF-Y (Krstic et al., 2007). Therefore, the *SOX3* gene is part of the emerging class of genes harboring multiple functional CCAAT boxes within their regulatory regions (Colter et al., 2005; Salsi et al., 2003).

The majority of promoters contain only one CCAAT box (either in the forward or reverse orientation) present at positions between -60 and -100 (Mantovani 1998). This has been found to be the average distance from the *tsp* for functional CCAAT boxes in promoters that lack a TATA box (Mantovani 1998), such as *Sox2/SOX2*. Namely, the characterization of the murine *Sox-2* promoter has revealed the presence of a consensus inverted CCAAT box motif that can bind the trimeric transcription factor NF-Y from nuclear extracts of murine F9 EC cells, and whose mutation significantly reduces the expression of *Sox-2* promoter/reporter constructs (Wiebe et al., 2000).

When we overexpressed NF-Y subunit A in NT2/D1 cells, only a mild positive effect on human SOX2 expression was detected (Fig. 1A). This result led us to propose that it is very likely that, in addition to NF-Y, other TFs are necessary to enable the proper activation of the *SOX2* promoter. One of the possible candidates might be the specificity protein 1 (Sp1). Sp1 is an ubiquitous, zinc-finger, transcription factor that binds GC/GT-rich promoter elements (Kadonaga et al., 1987). Activation of genes by Sp1 is highly complex, and is dependent on the gene promoter, cell context and interactions with other nuclear proteins (Pugh and Tjian, 1990).

Regarding the human *SOX3* gene, we have shown that the Sp1 site within its minimal promoter region is of functional relevance for its constitutive expression in NT2/D1 cells (Kovacevic

Grujicic et al., 2005). Here we have shown that in undifferentiated NT2/D1 cells overexpressed Sp1 caused a significant increase in the SOX2 protein level (Fig. 1B).

There are many examples of promoters that require the concerted action of Sp1 and NF-Y (discussed in Clem et al., 2003), both within a minimal promoter region of approx. 100 bp upstream of the *tsp* (Benfante et al., 2005) and the extended promoter regulatory region (approx. 600 bp) (Magan et al., 2003). Since the human *SOX2* promoter does not contain a TATA element, it is possible that this gene also requires the co-operative functional interaction between NF-Y and Sp1 in order to sustain its transcription. Moreover, since both NF-Y and Sp1 are ubiquitously expressed, it is quite possible that by enabling protein-protein interactions they co-operate in recruiting one or more neuro-specific co-activators, which subsequently activate the transcription of the *SOX2* gene in a tissue-specific fashion in NT2/D1 cells.

Effect of transcription factor MAZ

We also included in our analysis the Myc-associated zinc finger protein (MAZ). This protein was first identified as a TF bound to the promoter of the c-myc gene and to a sequence that is involved in the termination of transcription of the complement 2 gene (Bossone et al., 1992). MAZ appears to have a dual role in the initiation and termination of transcription (Ashfield et al., 1994; Parks and Shenk, 1997). This TF is expressed ubiquitously, but at different levels in different human tissues, and its expression appears to be regulated in a cell cycle-dependent manner by the MAZ protein itself (Song et al., 1998).

Recently, we have demonstrated that two out of three potential MAZ binding sites within the human SOX3 promoter can specifically bind the MAZ protein (Kovacevic Grujicic et al., 2008). Furthermore, we have shown that MAZ acts as a positive regulator of SOX3 gene transcription in undifferentiated NT2/D1 cells. Here we revealed that MAZ has the same effect on the SOX2 expression. Namely, as shown in Fig. 1B, overexpression of MAZ led to the up-regulation of the endogenous SOX2 protein level.

Taken together, here we report that overexpression of ubiquitous TFs NF-Y, Sp1 and MAZ resulted in a mild to significant activation of the human SOX2 expression (fold increase from 1.4 to 2.5; Fig. 1A and B) in the NT2/D1 model system.

Effect of TALE transcription factors on SOX2 expression

The TALE (three amino acid loop extension) subclass of homeodomain proteins regulates transcription of many essential developmental genes during embryogenesis, acting as either transcriptional activators or repressors (Marquardt, 2003). This super-family of DNA-binding proteins includes members of the PBX, MEIS, TGIF and PKNOX protein families (diIorio et al., 2007).

PBX1 (pre-B cell leukemia transcription factor 1) belongs to the PBX family of transcription factors. They are proposed to act as central developmental factors with a role in integrating transduction signals (Laurent et al., 2008). Namely, it has been shown that PBX proteins functionally interact with a wide variety of TFs by forming heterodimers, heterotrimers or multimers on the promoters of target genes that, like *SOX2*, play key roles in cell fate (reviewed in Laurent et al., 2008).

Human MEIS1 (myeloid ecotropic viral integration site 1 homolog) has been cloned from BMH-2 myeloid tumors as a novel common oncogenic site of proviral integration (Moskow et al., 1995). In *Xenopus laevis*, Meis1b regulates hindbrain gene expression (Maeda et al., 2002), while the ectopic expression of Meis caudalizes neural cell fates (Salzberg et al., 1999).

In contrast to the activation role of PBX1 and MEIS1 proteins, TGIF (5′-TG-3′ interacting factor) functions as a transcriptional repressor (Bertolino et al., 1995).

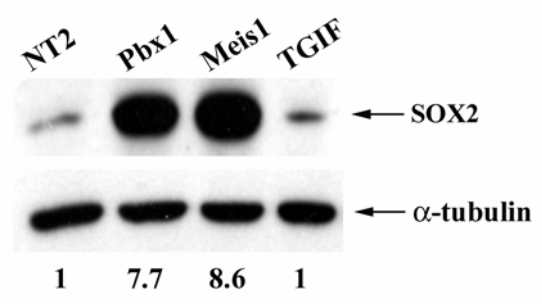

Fig. 2. Effect of TALE TFs on SOX2 protein expression. Western blot analyses of WCLs from NT2/D1 cells, non-transfected, or transfected with expression vectors for Pbx1, Meis1 or TGIF were performed using antibodies specific for SOX2 and α-tubulin, as indicated on the right. Quantitation of SOX2 protein level (relative to non-transfected NT2/D1 cells which was set as 1) is shown below.

Using the NT2/D1 model system, we have shown that Pbx1 and Meis1 proteins up-regulate *SOX3* gene expression. Also, overexpression of Pbx1 and Meis1 increases endogenous SOX3 protein expression (Mojsin and Stevanovic, 2009). Contrary to this, TGIF acts as a transcriptional repressor of the *SOX3* gene in NT2/D1 cells (Mojsin, 2008). Results presented here clearly demonstrate that Pbx1 and Meis1 cause the same effect on the SOX2 expression, i.e., overexpression of each of these two TFs in un-induced NT2/D1 cells causes considerable up-regulation of the endogenous SOX2 protein level (Fig. 2). On the other hand, the TGIF transcription factor does not cause any significant effect on the SOX2 expression in the same model system (Fig. 2).

Effect of liganded retinoid X receptor on SOX2 expression

Retinoic acid (RA) is a naturally occurring vitamin A derivative that regulates a broad range of biologic processes, with an essential role in neurodevelopment (Maden, 2002). RA mainly exerts its pleiotropic effects through the two families of nuclear retinoid receptors: RA receptors (RARs; NR1B) and retinoid X receptors (RXRs; NR2B), each containing three different receptor types: α, β, and γ (Mangelsdorf et al., 1995). In response to retinoid binding, members of these two receptor families form stable heterodimers that modulate the transcription of target genes by interacting with *cis*-acting RA response elements (RAREs) (Laudet and Gronemeyer, 2002). Also, RXR acts as an obligatory heterodimeric partner for a various array of other members of the intracellular receptor superfamily (Laudet and Gronemeyer, 2002).

We have shown previously that the human *SOX3* gene is expressed at low level in undifferentiated NT2/D1 cells, and that a 48 h exposure to RA leads to the up-regulation of its expression at both mRNA and protein levels (Mojsin et al., 2006; Nikcevic et al., 2008; Stevanovic, 2003). Furthermore, we have shown that RXRs are the major mediators of RA effect on the activation of *SOX3* gene expression. On the other hand, for the *SOX2* gene, a relatively high level of expression is detected in undifferentiated NT2/D1 cells, which does not significantly change following RA treatment (Deb-Rinker et al., 2005).

Regardless of the fact that the expression patterns of the *SOX2* and *SOX3* genes are different during the early phases of RA induced differentiation of NT2/D1 cells, we decided to test the response of the *SOX2* gene to overexpression of liganded RXR. As expected, a 48 h treatment of NT2/D1 cells by RA solely did not cause any significant effect on the SOX2 protein level (Fig. 3). However, when the nuclear receptor RXRα has been overexpressed in the presence of RA a significant increase in SOX2 expression has been detected (Fig. 3). The observed activation might be the result of a direct interaction of RXRα with control element(s) within the *SOX2* regulatory region. Also, besides this direct mediation of RA activity, many other indirect effects of RA might be responsible for triggering the SOX2 up-regulation under the described conditions. One of them, for example, could be linked to the Wnt signaling pathway. Namely, it has been shown that RA treatment leads to a decrease in the activity of the Wnt signaling, i.e. the TCF/LEF-mediated transactivation, in several cell lines (Eas-

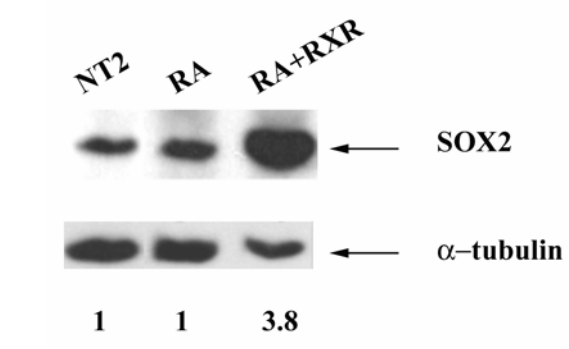

Fig. 3. Liganded RXRα up-regulates human SOX2 protein expression. Western blot analyses of WCLs from untreated and RA-treated NT2/D1 cells, either in the absence or presence of RXRα expression vector, were performed using antibodies specific for SOX2 and α-tubulin, as indicated on the right. Quantitation of SOX2 protein level (relative to non-transfected NT2/D1 cells which was set as 1) is shown below.

waran et al., 1999). Furthermore, it has been shown that Tcf3, a member of the Tcf/Lef family and a component of the Wnt signaling pathway, partially reduces expression of the three pluripotency regulators, *Oct4*, *Sox2*, and *Nanog* in mES cells (Cole et al., 2008). Therefore, we can speculate that RA treatment of NT2/D1 cells in the presence of excess nuclear retinoid receptor could lead to a reduced activity of TCF signaling, which in turn would cause the partial relief of the suppression of the *SOX2* gene expression, resulting in an increase of the SOX2 protein level. It is interesting to point out that previous comparative *in silico* analysis revealed a conserved TCF/LEF binding site among human, rat and mouse *Sox2* promoters (Katoh and Katoh, 2005).

In silico analysis of the human SOX2 promoter

Despite the fact that a number of far-distant regulatory elements and corresponding TFs responsible for the *SOX2* gene expression have been identified, it is not entirely revealed how the expression of this gene is regulated. Previous functional analyses of the mouse *Sox2* promoter revealed that the region from -528 to +238 relative to the transcriptional start point (*tsp*) contains regulatory elements capable of activating gene transcription (Wiebe et al., 2000) (Fig. 4a). It has also been shown that a smaller region, located between -426 to +9, is able to regulate *Sox2* gene expression (Lengler et al., 2005), (Fig. 4b). However, the promoter region of the human *SOX2* gene has not been characterized.

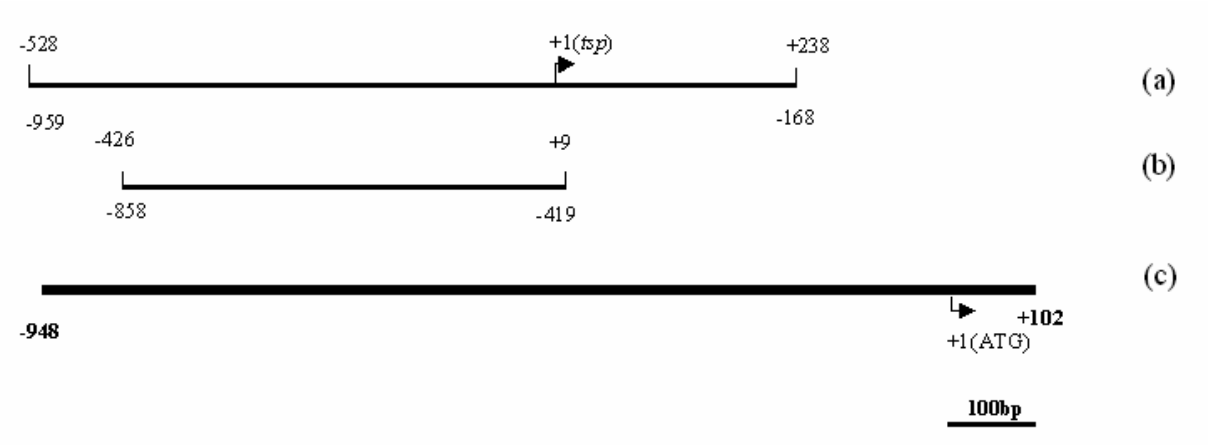

Fig. 4. Schematic overview of *Sox2/SOX2* promoters. (a) Murine *Sox2* promoter analyzed by Wiebe et al., 2000. (b) Murine *Sox2* promoter analyzed by Lengler et al.(2005). (c) Human *SOX2* promoter predicted by PromoterInspector software. Numbers above fragments indicate the end positions of promoter regions relative to murine *tsp* that is designated as +1 (*tsp*). Numbers below fragments indicate the end positions of promoter regions relative to adenosine in the first ATG codon of the human *SOX2*, denoted as +1 (ATG).

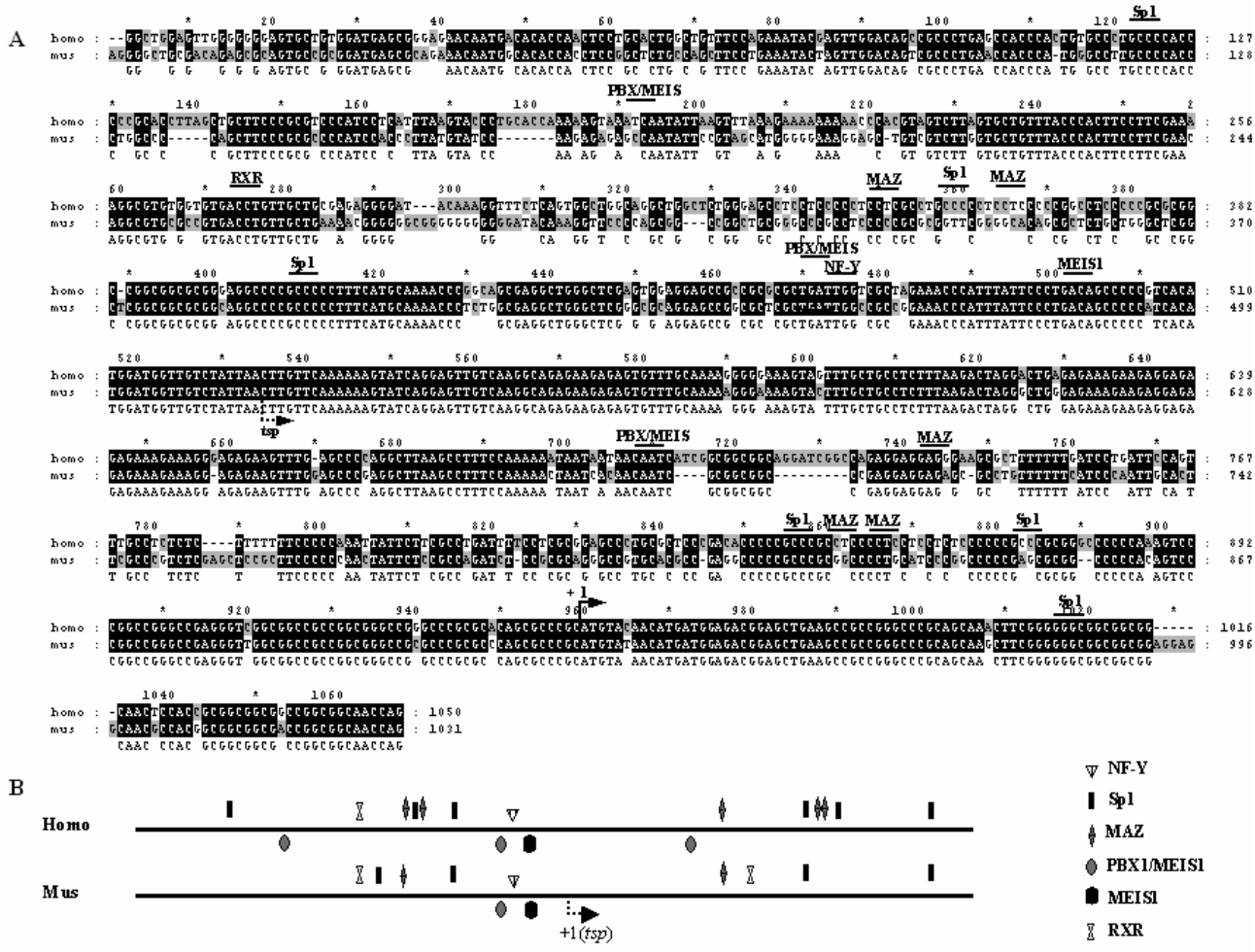

Fig. 5. (A). *In silico* analysis of human and mouse *SOX2/Sox2* promoter regions. The binding sites for transcription factors, previously shown to be involved in the regulation of human *SOX3* gene expression (NF-Y, Sp1, MAZ, PBX, MEIS and RXR) are indicated above the aligned sequences. The translational start point is designated as +1 and represented by filled arrow. Murine *Sox2 tsp*, as determined by Wiebe et al., (2000) is represented by dotted arrow. Homo-human (*Homo sapiens*); Mus-mouse (*Mus musculus*).

Based on data described by Wiebe et al., (Wiebe et al., 2000), we performed the bioinformatic analysis of the 5'-flanking region of the human *SOX2* gene (human genome sequence NT_005612.16). The PromoterInspector software revealed that the predicted *SOX2* promoter is positioned 948 bp upstream and 102 bp downstream from the adenosine in the first ATG codon denoted as +1 (nucleotide position from 87924351 to 87925400 of human genome sequence NT_005612.16) (Fig. 4c).

Our next goal was to compare the predicted human *SOX2* promoter sequence with the corresponding 5' flanking sequence of the mouse orthologue using a bioinformatics tools. Namely, we sought to identify the evolutionary conserved motifs and important *cis*-regulatory elements between the promoter regions of *SOX2/Sox2* genes with respect to factors which we have shown to be relevant to human *SOX3* and *SOX2* gene expression (NF-Y, Sp1, MAZ, Pbx1, Meis1 and RXRα). Alignment of the promoter sequences of

SOX2/Sox2 genes using ClustalW and GeneDoc software revealed a significant level of their conservation during evolution (80% sequence identity) (Fig. 5A). Furthermore, results of Matlnspector analysis pointed to a cluster of four TFs (Sp1, PBX1, NF-Y and MEIS1), closely located to the mouse *tsp* that are highly conserved in sequence and in position, among the analyzed human and mouse promoters (Fig. 5A and B). Also, an up-stream RXR binding site is conserved, as well as two Sp1 binding sites down-stream from the mouse *tsp* (Fig. 5A and B). This *in silico* analysis supports our Western blot results showing the involvement of selected TFs in the regulation of SOX2 expression, particularly since it has been postulated that non-coding sequences that can be aligned across two or more organisms could have functional regulatory roles (Boffelli et al., 2003). Apart from the conserved sites, our analysis revealed some differences in the number and position of putative binding sites for transcription factors Sp1, MAZ, Pbx1 and RXR (Fig. 5A and B).

Taken together, we have presented data indicating that some aspects of the transcriptional regulation of *SOX2* and *SOX3* genes are preserved. Similarly to SOX3, we report here on the activation of SOX2 expression by ubiquitous TFs (NFY, Sp1 and MAZ) by TALE family members (Pbx1 and Meis1), as well as liganded RXRα. On the other hand, TGIF is the only analyzed TF whose overexpression in NT2/D1 cells caused a different response in SOX3 and SOX2 expression. Namely, TGIF acted as a transcriptional repressor of the *SOX3* gene, while no significant effect of this TF on the SOX2 expression has been revealed in this study.

Data presented here could help in a better understanding of the molecular mechanism(s) responsible for the regulation of expression of the *SOX2* gene in a human EC model system. This line of research is very important, given the essential role of SOX2 in diverse developmental settings, both physiological and pathological. Namely, it has been shown that SOX2 is implicated in the normal development of the brain and the pituitary gland, as well as of the eyes and inner ear (Kelberman et al., 2006). Also, it has been reported that heterozygous *SOX2* mutations in humans cause hippocampal defects, forebrain abnormalities and anophthalmia (Kelberman et al., 2006).

Therefore, the knowledge gained by studying genes that participate in maintaining or modifying ES/EC cell states, such as *SOX2*, could open up new possibilities in therapies, particularly those related to the replacement of lost cells, either by cell transplantation or by the manipulation of a patient's progenitor cells *in situ*. Since the experimental model used in this study are NT2 EC cells, it is worth highlighting that they have been used for transplantation as cell therapy for brain injury, ischemia and neurodegenerative diseases in animal models, as well as in two clinical trials with human stroke patients (reviewed in Newman et al., 2005). In that respect, we believe that elucidating components involved in the regulation of *SOX* genes expression, and *SOX2* in particular, in a human EC model system could provide a valuable contribution to this field.

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УТИЦАЈ ОПШТИХ ТРАНСКРИПЦИОНИХ ФАКТОРА, TALE ПРОТЕИНА И ЛИГАНДОМ АКТИВИРАНОГ НУКЛЕАРНОГ РЕЦЕПТОРА RXRα НА РЕГУЛАЦИЈУ ЕКСПРЕСИЈЕ ХУМАНОГ SOX2 ГЕНА У ЕМБРИОНАЛНОЈ КАРЦИНОМСКОЈ NT2/D1 ЋЕЛИЈСКОЈ ЛИНИЈИ

МИЛЕНА МИЛИВОЈЕВИЋ, ГОРДАНА НИКЧЕВИЋ, НАТАША КОВАЧЕВИЋ-ГРУЈИЧИЋ, А. КРСТИЋ, МАРИЈА МОЈСИН, ДАНИЈЕЛА ДРАКУЛИЋ и МИЛЕНА СТЕВАНОВИЋ

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SOX2 транскрипциони фактор има кључну улогу у процесима ембрионалног развића и представља универзални маркер плурипотентних матичних ћелија. С обзиром на функционалну редундантност и преклапајући профил експресије чланова SOXB1 подгрупе током развића, циљ овог рада био је да испита потенцијалне заједничке аспекте регулације експресије *SOX2* и *SOX3* гена. Наиме, испитиван је утицај одабраних транскрипционих фактора на регулацију експресије *SOX2* гена у NT2/D1 ћелијској линији. Анализирани су они фактори за које је претходно показано да су укључени у модулацију активности хуманог *SOX3* гена. Резултати ових истраживања указују да општи транскрипциони фактори (NF-Y, Sp1 и MAZ), чланови TALE фамилије протеина (Pbx1 и Meis1), као и ретиноичном киселином активиран нуклеарни рецептор RXRα доводе до повећане експресије SOX2 протеина. Испитивање транскрипционих фактора укључених у регулацију експресије *SOX* гена је значајно за боље разумевање сигналних путева који су активни у плурипотентним матичним ћелијама.