Biochemical and Biophysical Research Communications 412 (2011) 86–91

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Identification of a novel Sp1 splice variant as a strong transcriptional activator

Vittoria Infantino [a,b,1], Paolo Convertini [a,1], Francesco Iacobazzi [a], Isabella Pisano [a], Pasquale Scarcia [a], Vito Iacobazzi [a,c,*]

[a] Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Bari, Italy
[b] Department of Chemistry, University of Basilicata, Potenza, Italy
[c] CNR Institute of Biomembranes and Bioenergetics, Via Orabona 4, 70125 Bari, Italy

ARTICLE INFO

Article history:
Received 6 July 2011
Available online 22 July 2011

Keywords:
Sp1
Splice variant
Transcription
Gene expression
Activator
Mitochondrial citrate carrier
Mitochondrial carnitine carrier

ABSTRACT

The transcription factor Sp1 regulates expression of numerous genes involved in many cellular processes. Different post-transcriptional modifications can influence the transcriptional control activity and stability of Sp1. In addition to these modifications, alternative splicing isoforms may also be the basis of its distinct functional activities. In this study, we identified a novel alternative splice isoform of Sp1 named Sp1c. This variant is generated by exclusion of a short domain, which we designate α, through alternative splice acceptor site usage in the exon 3. The existence of this new isoform was confirmed *in vivo* by Western blotting analysis. Although at very low levels, Sp1c is ubiquitously expressed, as seen in its full-length Sp1. A preliminary characterization of Sp1c shows that: (a) Sp1c works as stronger activator of transcription than full-length Sp1; (b) percentage of HEK293 Sp1c-overexpressing cells is higher in G1 phase and lower in S phase than percentage of HEK293 Sp1-overexpressing cells.

1. Introduction

Sp1, a member of the Sp-multigene family (Cys_2His_2-type zinc finger transcriptional factors) [1], is probably one of the best characterized sequence-specific transcription factors. Through its binding to GC boxes, Sp1 regulates the expression of TATA-less and TATA-containing genes via protein–protein interaction. Combination of oligonucleotides arrays and chromatin immunoprecipitation has led to the estimation that the human genome contains at least 12,000 Sp1 binding sites [2]. Therefore, it is not surprising that Sp1 has been implicated in the expression of numerous genes involved in many aspects of cellular life, such as metabolism, cell growth [1], differentiation [3], and apoptosis [1]. Sp1 binds to the promoters of many genes and its activity is modulated by a variety of stimuli through post-transcriptional modification. Phosphorylation, acetylation, sumoylation, ubiquitylation, and glycosylation are among the post-translational modifications that can influence the transcriptional control activity and stability of Sp1 [4]. Although Sp1 mRNAs, proteins and sequence specific DNA-binding motifs are widely found, Sp1 activation has been implicated in mediating developmental-specific gene expression [5]. This disagreement clearly involves one or more regulatory mechanisms mentioned above, but additional conditions may also be of importance, including regulated expression of splicing isoforms with distinct functions [6]. In fact, it has been estimated that 40 ± 60% of the human gene transcripts are alternatively spliced [7] and the amount of alternative splicing in a given gene appears to be dependent on the function. Among these, genes involved in transcription regulation are more frequently subject to alternative splicing [8]. In this regard Sp3, a member of the Sp-multigene family, gives rise 3 splice isoforms generated by alternative transcriptional initiation site [9a].

Here we identify a novel Sp1 splice isoform (Sp1c) due to α domain exclusion through alternative splice acceptor site usage in the exon 3. Moreover, we describe Sp1c expression pattern and its initial functional characterization.

2. Materials and methods

2.1. Construction of plasmids

The pGL3-CIC and the pGL3-CAC were obtained as described previously [10,11]. The full-length coding regions of the human Sp1a and Sp1c variants were amplified from HEK293 cDNA using the iProof High Fidelity DNA Polymerase (Bio-Rad), the forward primer 5′-ACAAAGCTTATGAGCGACCAAGATCACTCCA-3′ and the reverse primer 5′-GAGGAATTCTCAGAAGCCATTGCCACTGATAT-3′. The Sp1a and Sp1c cDNAs were cloned into HindIII/EcoRI sites of a pcDNA3.1 (+) expression vector (Invitrogen). The resulting vectors were termed pcDNA3-Sp1a and pcDNA3-Sp1c. The plasmid

* Corresponding author at: Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Via Orabona 4, 70125 Bari, Italy. Fax: +39 080 544 2770.
E-mail address: viacob@farmbiol.uniba.it (V. Iacobazzi).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.07.047

DNAs were isolated by using the High Pure Plasmid Isolation Kit (Roche). The fidelity of the final inserts in pcDNA3 plasmid was verified by DNA sequencing using BigDye Terminator Kit (Applied Biosystems) and the following primers: pcDNAF primer (5′-AATACGACTCACTATAGGGA-3′), Sp1-600F (5′-TCTGGTCAAATACAGATC-3′), Sp1-1100F (5′-CAGTGGGCTACAGGGGTCTGA-3′), Sp1-1700F (5′-AATACATGATGACACAGCA-3′), or pcDNAR primer (5′-AGAAGGCACAGTCGAGGC-3′).

2.2. Cell culture, preparation of total and nuclear extracts, and transient transfection

HEK293 cells (Sigma) were maintained as described [12]. For HEK293 total extracts, cells were washed twice in cold PBS (137 mM NaCl, 2.7 mM KCl, 1.9 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) and then lysed in RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate, protease inhibitors, 0.1% SDS) for 1 h on ice. Lysates were then cleared by centrifugation at 10,000*g* for 15 min at 4 °C. For nuclear extracts preparation, HEK293 cells (1×10^7) were washed once with ice-cold phosphate-buffered saline (PBS), and lysed for 30 min in ice-cold lysis buffer (10 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (Hepes), pH 7.9, 0.1 mM ethylenediaminetetraacetate (EDTA), 1 mM dithiothreitol (DTT), 1 mM PMSF, 1 mM aprotinin, 1.5 mM leupeptin, 0.5% Triton X-100). Cell lysates were centrifuged for 10 min at 4 °C (10,000*g*). The pellet was resuspended in the high salt lysis buffer (20 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM PMSF, 0.1% aprotinin), and incubated at 4 °C for 2 h, then centrifuged for 5 min at 4 °C (10,000*g*). The resulting supernatants were reserved as nuclear extracts. The protein concentrations of HEK293 total and nuclear extracts were determined by Bradford protein assay (Bio-Rad). Transient transfection was performed, as reported in Iacobazzi et al. [13], using 0.5 μg of pGL3-CIC or pGL3-CAC reporter vector. The extent of transfection was normalized by β-galactosidase activity [14]. For overexpression, HEK293 cells were transfected with pcDNA3-Sp1a or pcDNA3-Sp1c.

2.3. RT-PCR and real-time PCR

RNA from HEK293 cells was extracted using GenElute Mammalian Total RNA Miniprep Kit (Sigma) according to the manufacturer's instructions. RNAs from human tissues were purchased from Ambion. cDNAs were synthesized from 1 μg of each RNA using GeneAmp RNA PCR Core kit (Applied Biosystem). Reverse transcriptase-polymerase chain reaction (RT-PCR) experiments were carried out using 1 μl of each cDNA as template and primers corresponding to +1/+22 (5′-ATGAGCGACCAAGATCACTCCA-3′) and +422/+441 (5′-ATACTGCCCACCAGAGACTG-3′) of the Sp1a CDS (CoDing Sequence). PCR was run in the exponential region to allow semi-quantitative comparisons among cDNAs developed from identical reactions. Real-time PCR was conducted as previously described [15]. Assay-on-demand for human CIC (Hs00761590_m1) and human β-actin (4326315E) were purchased from Applied Biosystems. CIC transcript levels were normalized against the β-actin expression levels.

2.4. Western blotting

Thirty micrograms of the HEK293 total or nuclear extracts was heated at 100 °C for 5 min, separated on 4–12% SDS polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were then blocked for 1 h in a PBS solution containing 2% bovine serum albumin and 0.1% Tween 20, and then treated at room temperature with anti-Sp1 (Santa Cruz Biotechnology) or anti-β-actin (Santa Cruz Biotechnology) antibodies. The immunoreaction was detected by the Immobilon Western ECL system (Millipore).

2.5. Cell count and flow cytometry

Cells were detached from the plate using a 0.05% trypsin–EDTA solution (Sigma) for 5 min at 37 °C. Equal volumes of HEK293 cell suspension (10 μl) and 0.4% Trypan blue (Sigma) in PBS were mixed, and the cells were scored under phase contrast microscope using a haemocytometer. Percentage survival was calculated as percentage of live cells divided by total cell number (including dead and live cells). For cell-cycle analysis, 1×10^6 HEK293 cells were fixed in 70% ethanol, incubated for 30 min at 4 °C, treated with RNase A (500 μg/ml), and stained with propidium iodide 0.46 μM (Sigma) for 30 min at 37 °C. Cell cycle analysis was performed on a flow cytometer (EPICS® Elite; Backman Coulter).

3. Results

3.1. Identification of a novel Sp1 human splice variant

In order to investigate the Sp1 effect on mitochondrial carrier gene transcription [10,11], we amplified Sp1 CDS from various cell lines. Surprisingly, PCR from HEK293 cDNA revealed two bands (Fig. 1A). When sequenced, we found that the longest fragment was the full-length human Sp1, instead the shortest one revealed a 144-bp deletion (from 163 to 306 bp), in coding sequence compared to full-length Sp1. This deletion extends the length of the intron 2 from 296 to 440 bp (Fig. 1B). We designated as α the region excluded in Sp1 CDS by use of this alternative cryptic splice acceptor within exon 3. The nucleotide sequence of the new splice variant, that we named Sp1c, was deposited in GenBank (Accession No. FN908228). Beyond the known isoform Sp1a (wild type), another isoform Sp1b (Accession No. NM_003109) has also been identified but not functionally characterized. Sp1b contains an alternative exon in the 5′ coding region and uses a downstream start codon, compared to Sp1a (Fig. 1C). Sp1c, like Sp1a, contains the three activation domains (A, B, and C), the three Cys_2His_2 zinc "fingers", requested for the binding to GC-rich promoter elements and the carboxyl-terminal domain (D), needed for the synergistic activation [16], but it is missing of a part of the inhibitory domain (Fig. 1D). In order to reinforce our results, computer alignment by the BLAST (http://blast.ncbi.nlm.nih.gov) of the Sp1c CDS from + 1 to + 500 bp (encompassing the splicing region) was performed. This analysis revealed the presence of an expressed sequence tag in embryonal kidney *Homo sapiens* cDNA (BP872136), suggesting that α domain could be excluded from Sp1 CDS, i.e., included in the intron 2 (Fig. 1B).

3.2. Expression of Sp1c

To confirm the *in vivo* existence of Sp1c isoform, Western blotting experiments were carried out on total cell lysate and nuclear extracts from HEK293 cells. We used an antibody that recognizes a region from 121 to 345 amino acids of the Sp1a, a region also present in Sp1c. Immunodecoration of the whole HEK293 cell lysate failed to reveal the Sp1c isoform (Fig. 2A). When the same experiment was performed on nuclear extracts of the same cell line two bands, corresponding to the Sp1a and the newly identified Sp1c, were observed (Fig. 2A). The discovery of the new splice variant of Sp1, led us to search for tissues where this splicing event takes place. For this purpose we used RT-PCR with total RNA from different human tissues as templates. Since it has been reported that Sp1 is widely expressed, nineteen human tissues were screened for the presence of Sp1c and Sp1a transcripts

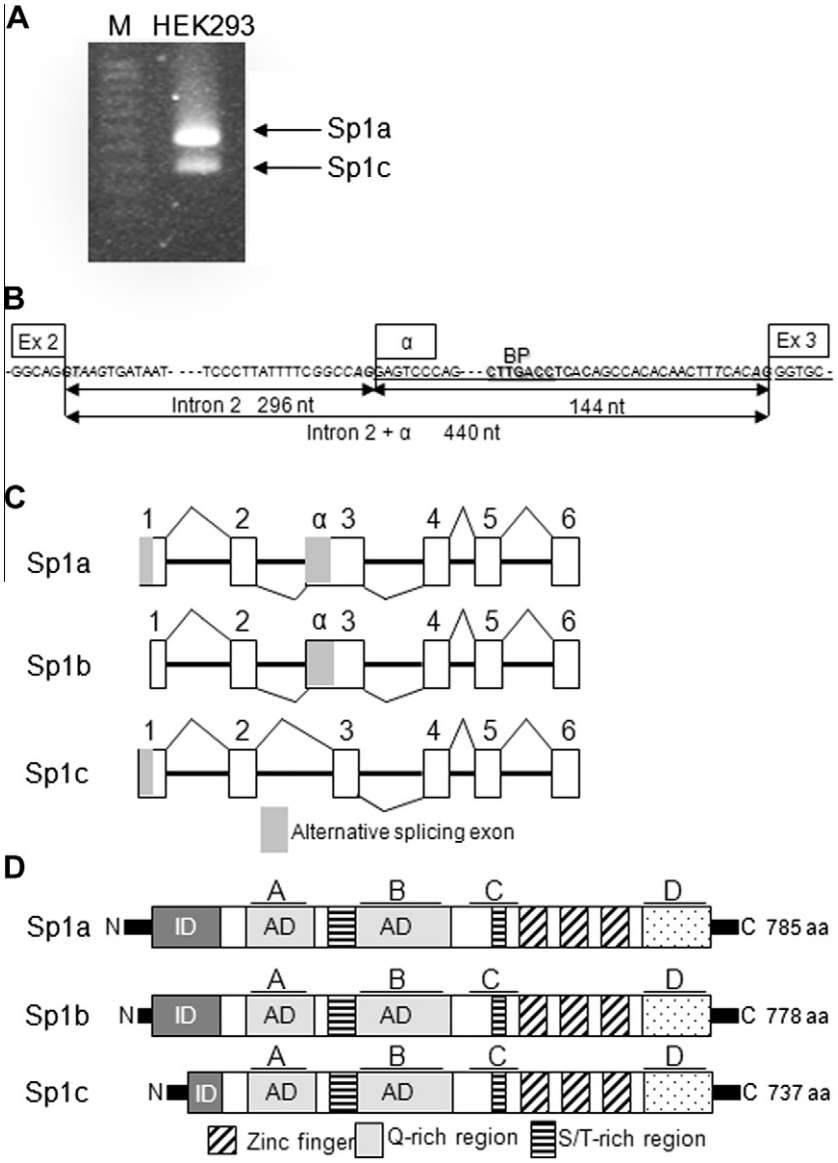

Fig. 1. Human Sp1 gene. (A) RT-PCR of total RNA from HEK293 cells identifies the Sp1a (441 bp) and Sp1c (297 bp) transcripts. (B) Partial exon–intron structure in the region of the Sp1 gene containing exon 2, intron 2, α domain, and exon 3. Dashed lines represent intron sequence. The length of the intron 2, α domain and intron 2 + α domain of the Sp1 gene are indicated. BP, branch point. (C) Schematic representation of human Sp1 gene structure: Sp1a and alternative splicing events, Sp1b and Sp1c, are indicated. Open boxes represent exons. The alternative splicing exons are in light gray. (D) Structural domains of Sp1. Amino acid length of the Sp1 isoforms are indicated on the right. The black bars marked with (A–D) denote the sub-domains. (A–C) Domains represent the activation region. AD, activation domain; ID, inhibitory domain.

(Fig. 2B). The primers, used to detect α domain isoforms, spanned the Sp1 exon 1–4 (+1/+441 bp) of Sp1a CDS. PCR analysis showed two transcripts. The first of about 440 bp corresponded to Sp1a and the second one of about 300 bp to Sp1c (Fig. 2B). These results showed that α− mRNAs were present in all tissues, although were much less abundant than α + mRNAs (Fig. 2B). This finding could explain the inability to identify the Sp1c protein in whole cell lysates.

3.3. Transcriptional control activity by Sp1c

Since Sp1 is a transcription factor, we tested Sp1c transactivation ability on two Sp1-responsive promoters belonging to the mitochondrial carrier family, the citrate carrier (CIC) or the carnitine/acylcarnitine carrier (CAC) gene [9b,10,11]. Luciferase activity of pGL3-CIC and pGL3-CAC reporter constructs was measured in HEK293 Sp1c-overexpressing cells (Fig. 3A and B, respectively). Sp1c transcriptional activity was compared to Sp1a of HEK293 Sp1a-overexpressing cells, also transfected with pGL3-CIC and pGL3-CAC (Fig. 3A and B, respectively). Sp1c overexpression caused a significantly increase of transcriptional activity by about nine (Fig. 3A) and threefold (Fig. 3B) on CIC and CAC gene promoters, respectively, as compared to controls. Interestingly, CIC promoter, containing five functional Sp1-responsive elements [10], was more activated than CAC promoter which has only one functional Sp1 target site [11]. Moreover, the Sp1c transactivation increased by about 40% and 25% on CIC and CAC gene promoters, respectively, when compared to Sp1a (Fig. 3A and B). In another set of experiments, HEK293 cells were cotransfected with different ratios of Sp1a (α+) and Sp1c (α-) in presence of pGL3-CIC. Expression of the α− form alone produced more than about 40% induction of gene reporter activity. The α + form alone was set to 100% (Fig. 3C). Finally, we tested Sp1c transactivation ability on endogenous Sp1-responsive CIC gene. Sp1c overexpression induced an

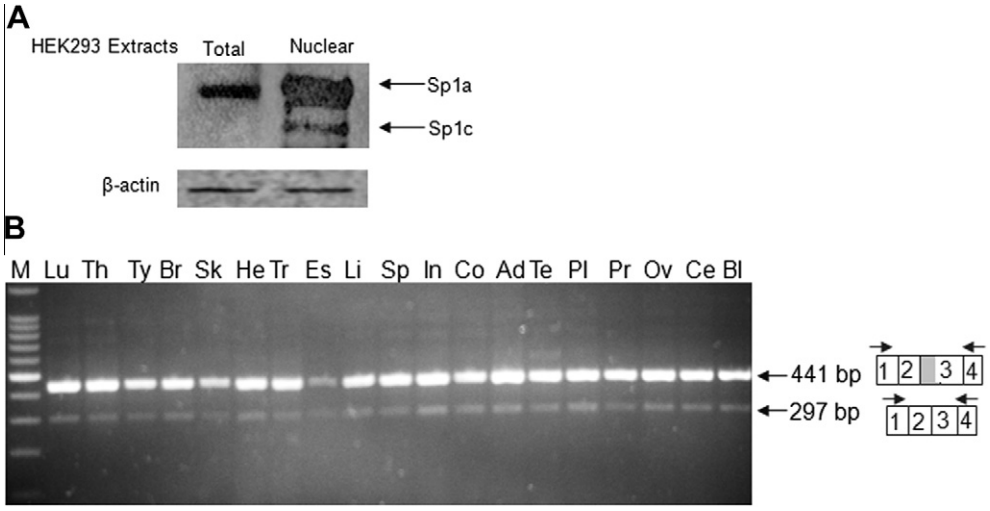

Fig. 2. Expression of Sp1c. (A) Sp1 and β-actin of HEK293 total or nuclear extracts were immunodecorated with specific antibodies. Arrows on the right indicate the position of Sp1a and Sp1c. (B) The RT-PCR analysis of spliced variants with RNAs from many human tissues is shown. On the right, a schematic representation of the portion of Sp1 transcripts. Primers used are indicated by arrows. Lu, lung; Th, thymus; Ty, thyroid; Br, brain; Sk, skeletal muscle; He, heart; Tr, tracheas; Es, esophagus; Li, liver; Sp, spleen; In, small intestine; Co, colon; Ad, adipose tissue; Te, testis; Pl, placenta; Pr, prostate; Ov, ovaries; Ce, cervix; Bl, bladder.

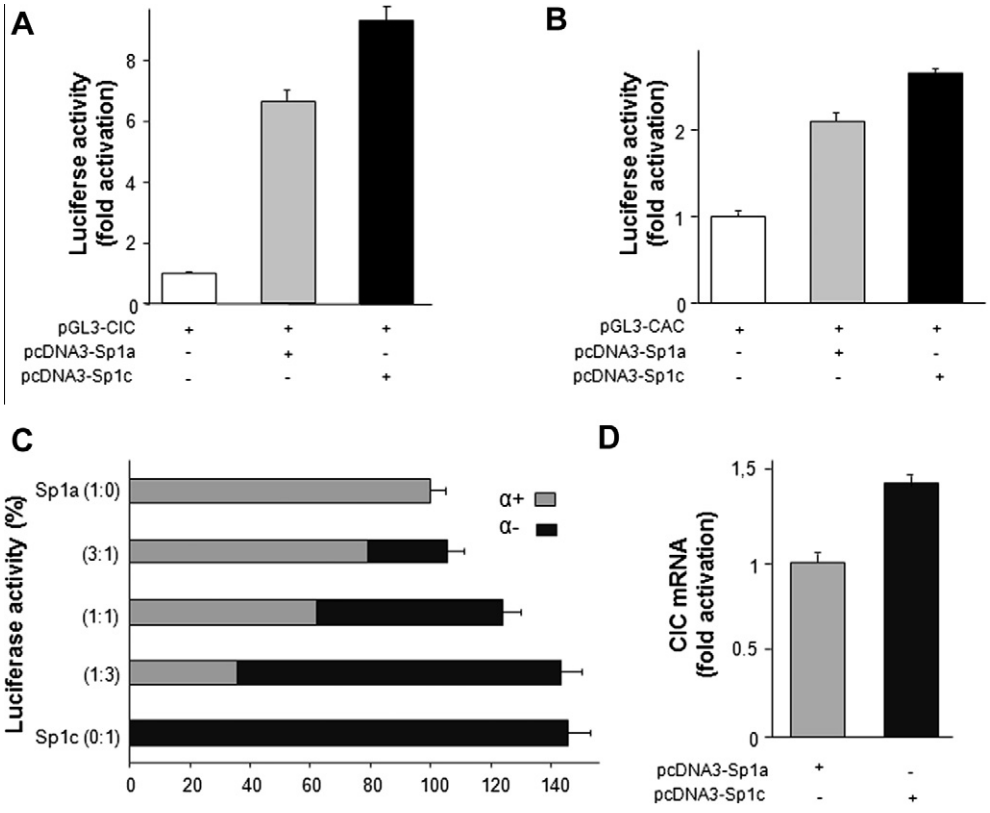

Fig. 3. Sp1c transcriptional control activity. (A,B) HEK293 cells were transiently cotransfected with the pcDNA3 empty vector (white bars), pcDNA3-Sp1a (gray bars) or pcDNA3-Sp1c (black bars) and the pGL3-CIC and pGL3-CAC, respectively. (C) HEK293 cells were cotransfected with pGL3-CIC and either pCDNA3 or the indicated ratios of the pcDNA3-Sp1a (gray bars) to the pcDNA3-Sp1c (black bars). (D) Total RNA extracted from HEK293 cells transfected with the pcDNA3-Sp1a (gray bars) or the pcDNA3-Sp1c (black bars), was used to quantify CIC mRNA. Data are shown as means ± SD of five duplicate independent experiments. Differences between samples and relative controls were significant ($P < 0.05$, one-way ANOVA). Differences between pcDNA3-Sp1a (gray bars) and pcDNA3-Sp1c (black bars) samples were also significant ($P < 0.05$, one-way ANOVA).

increase of CIC mRNA level by about 40% higher than Sp1a overexpression (Fig. 3D). Similar results to those presented in Fig. 3C and D were obtained measuring CAC mRNA (data not shown). These results clearly indicate that Sp1c is a stronger activator than Sp1a.

3.4. Sp1c and cell cycle progression

Because Sp1 activates key factors involved in cycle progression through G1 phase and entry into S phase [17], we investigated the effect of Sp1c on cell cycle progression. First of all, flow cytometry

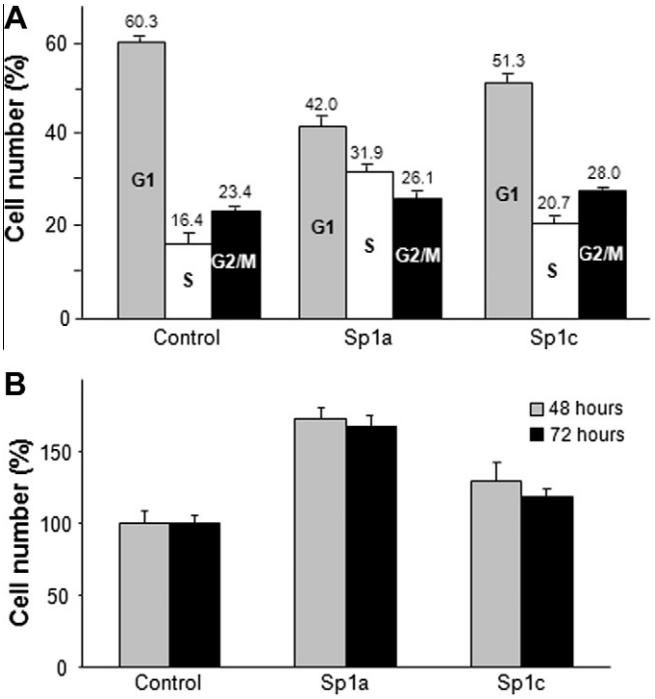

Fig. 4. Effect of Sp1c on cell cycle. (A) HEK293 cells were transfected with pcDNA3-Sp1a (Sp1a), pcDNA3-Sp1c (Sp1c) and pcDNA3 empty vector (Control). The percentage cell distribution (numbers above bars) in G1 (gray bars), S (white bars) and G2/M (black bars) phases was determined by flow cytometry analysis. (B) HEK293 cells transfected as described in (A) were counted. Data are shown as means ± SD of five duplicate independent experiments. Differences between samples and controls were significant ($P < 0.05$, one-way ANOVA). In (A) differences between Sp1c and Sp1a samples in G1 or S phase and in (B) differences between Sp1a and Sp1c samples were also significant ($P < 0.05$, one-way ANOVA).

analysis was performed. Although to a different extent, Sp1a and Sp1c determined a cell number decrease in G1 phase and an increment in S and G2/M phases compared to control (Fig. 4A). Moreover, the Sp1c overexpression caused a cell number increase and decrease in G1 phase and in S phase, respectively, compared to Sp1a-overexpressing cells. No significantly effects were observed on the cell percentage in G2/M phase between Sp1a-overexpressing cells and Sp1c-overexpressing cells (Fig. 4A). These results were in accordance with those of HEK293 cell count. The number of Sp1a- and Sp1c-overexpressing cells increased by about 60% and 20%, respectively, compared to controls as at 48 h as at 72 h. Otherwise, the number of Sp1c-overexpressing cells decreased by about 40% compared to the number of Sp1a-overexpressing cells (Fig. 4B). These preliminary data could suggest that Sp1c leads to lower cell cycle acceleration than Sp1a, probably by delaying cell cycle progression from G1 phase to S phase in our cellular model.

4. Discussion

Human Sp1 gene, localized on chromosome 12q13.1, spans a region of about 36 kbp and comprises 6 exons (GeneID: 6667) (Fig. 1C). Only one Sp1 mRNA variant, Sp1b, has been described until now which derives from usage of a differential start site.

In this work a novel Sp1 isoform is described. This novel variant is named Sp1c and it is generated by α domain exclusion through alternative splice acceptor site usage in the exon 3. Thus, Sp1c protein lacks 48 amino acids in the inhibitory domain compared to Sp1a (Fig. 1D). A comparison of the splice site used to generate Sp1c with a consensus splice site shows that the acceptor site contains a suboptimal poly-pyrimidine tract and a conserved AG dinucleotide (Fig. 1B). Moreover, the novel Sp1c exon starts with a G, as usually it occurs in the consensus sequence (Fig. 1B). A consensus branch-point (YNYURAY) [18] is located upstream of exon 3 (Fig. 1B, α domain), and according to the model where the splice acceptor site is identified by a scanning mechanism from the branch point, the first AG dinucleotide is preferred as the splice acceptor site [19]. It is noteworthy that the both the AG dinucleotide alternative acceptor site and the branch-point in α domain are present in other Sp1 genes sequenced, such as *Mus musculus*, *Xenopus laevis*, and *Gallus gallus*.

A preliminary Sp1c characterization shows that: (a) Sp1c is ubiquitously expressed in human tissues but at very low levels as indicated by RT-PCR experiments; (b) Sp1c causes a higher transcriptional upregulation than Sp1a, demonstrated both by gene reporter assay and endogenous Sp1-responsive gene activation; (c) Sp1c induces lower cell cycle acceleration than Sp1a as revealed by flow cytometry analysis. Even though the molecular mechanisms of these findings are not known currently, first of all it should be considered that most of the known examples (including Sp1c) are expressed at very low levels compared with the "normal" transcripts [20] indicating that these splicing events occur by usage of less efficient splice sites. Secondly, Sp1 phosphorylation state strongly affects its transcriptional activity through association with chromatin, conformational changes, recruitment of coactivators and repressors, and other post-translational modifications [4]. Two phosphorylation sites, serine 59 and serine 101, are missing in Sp1c. While serine 101 phosphorylation is involved in the DNA-damage stress response and does not affect its transcriptional activity [21], phosphorylation of serine 59 is needed in cell cycle progression [22]. It was reported that serine 59 is phosphorylated by cyclin-dependent kinase 2 (Cdk2) during the S phase of the cell cycle, and this results in transactivation of dihydrofolate reductase (DHFR), an essential event in the production of thymidine for DNA synthesis and cell cycle progression [23]. Finally, another aspect should be considered to explain the different behavior of Sp1a and Sp1c. Since lacking of 48 amino acids in Sp1c is located within inhibitory domain, we suppose that this domain is the most directly involved in determining the different transcriptional activity between Sp1a and Sp1c. In agreement with this hypothesis, Sp1c transcriptional upregulation was found higher than Sp1a, probably due to absence of a fully interaction with some corepressors, such as SMRT (silencing mediator of retinoid and thyroid receptor), NCor (nuclear hormone receptor corepressor) and BCoR (BCL-6 interacting corepressor), in the Sp1c inhibitory domain [24].

In summary, the present study highlights the existence of Sp1c, a novel human Sp1 splice variant. Sp1c displays a greater ability to activate the transcription compared to Sp1a. It is noteworthy that this important feature of Sp1c has been demonstrated not only through gene reporter assay but also by measuring the endogenous mRNA increase of Sp1-responsive genes. Next studies will be aimed to better clarify the biological meaning of Sp1c.

Acknowledgments

We thank prof. Ferdinando Palmieri for critically reading the original manuscript. This work was supported by grants from the University of Bari and The Italian Human ProteomeNet No. RBRN07BMCT_009.

References

[1] J. Kaczynski, T. Cook, R. Urrutia, Sp1- and Krüppel-like transcription factors, Genome Biol. 4 (2003) 206.
[2] S. Cawley, S. Bekiranov, H.H. Ng, P. Kapranov, E.A. Sekinger, D. Kampa, A. Piccolboni, V. Sementchenko, J. Cheng, A.J. Williams, R. Wheeler, B. Wong, J. Drenkow, M. Yamanaka, S. Patel, S. Brubaker, H. Tammana, G. Helt, K. Struhl, T.R. Gingeras, Unbiased mapping of transcription factor binding sites along

human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs, Cell 116 (2004) 499–509.
[3] O. Opitz, A. Rustgi, Interaction between Sp1 and cell cycle regulatory proteins is important in transactivation of a differentiation-related gene, Cancer Res. 60 (2000) 2825–2830.
[4] N. Tan, L. Khachigian, Sp1 phosphorylation and its regulation of gene transcription, Mol. Cell. Biol. 29 (2009) 2483–2488.
[5] K. Thomas, J. Wu, D. Sung, W. Thompson, M. Powell, J. McCarrey, R. Gibbs, W. Walker, SP1 transcription factors in male germ cell development and differentiation, Mol. Cell. Endocrinol. 270 (2007) 1–7.
[6] W. Walker, F. Delfino, J. Habener, RNA processing and the control of spermatogenesis, Front. Horm. Res. 25 (1999) 34–58.
[7] B. Graveley, Alternative splicing: increasing diversity in the proteomic world, Trends Genet. 17 (2001) 100–107.
[8] T. Ravasi, T. Huber, M. Zavolan, A. Forrest, T. Gaasterland, S. Grimmond, D. Hume, R.G. Group, G. Members, Systematic characterization of the zinc-finger-containing proteins in the mouse transcriptome, Genome Res. 13 (2003) 1430–1442.
[9] (a) A. Sapetschnig, F. Koch, G. Rischitor, T. Mennenga, G. Suske, Complexity of translationally controlled transcription factor Sp3 isoform expression, J. Biol. Chem. 279 (2004) 42095–42105;
(b) F. Palmieri, C.L. Pierri, A. De Grassi, A. Nunes-Nesi, A.R. Fernie, Evolution, structure and function of mitochondrial carriers: a review with new insights. Plant J. 66 (2011) 161–181.
[10] V. Iacobazzi, V. Infantino, F. Palmieri, Epigenetic mechanisms and Sp1 regulate mitochondrial citrate carrier gene expression, Biochem. Biophys. Res. Commun. 376 (2008) 15–20.
[11] P. Convertini, V. Infantino, F. Bisaccia, F. Palmieri, V. Iacobazzi, Role of FOXA and Sp1 in mitochondrial acylcarnitine carrier gene expression in different cell lines, Biochem. Biophys. Res. Commun. 404 (2011) 376–381.
[12] V. Iacobazzi, V. Infantino, P. Convertini, A. Vozza, G. Agrimi, F. Palmieri, Transcription of the mitochondrial citrate carrier gene: identification of a silencer and its binding protein ZNF224, Biochem. Biophys. Res. Commun. 386 (2009) 186–191.
[13] V. Iacobazzi, V. Infantino, F. Bisaccia, A. Castegna, F. Palmieri, Role of FOXA in mitochondrial citrate carrier gene expression and insulin secretion, Biochem. Biophys. Res. Commun. 385 (2009) 220–224.
[14] V. Iacobazzi, V. Infantino, P. Costanzo, P. Izzo, F. Palmieri, Functional analysis of the promoter of the mitochondrial phosphate carrier human gene: identification of activator and repressor elements and their transcription factors, Biochem. J. 391 (2005) 613–621.
[15] V. Iacobazzi, P. Convertini, V. Infantino, P. Scarcia, S. Todisco, F. Palmieri, Statins, fibrates and retinoic acid upregulate mitochondrial acylcarnitine carrier gene expression, Biochem. Biophys. Res. Commun. 388 (2009) 643–647.
[16] J.R. Davie, S. He, L. Li, A. Sekhavat, P. Espino, B. Drobic, K.L. Dunn, J.M. Sun, H.Y. Chen, J. Yu, S. Pritchard, X. Wang, Nuclear organization and chromatin dynamics – Sp1, Sp3 and histone deacetylases, Adv. Enzyme Regul. 48 (2008) 189–208.
[17] A. Black, J. Black, J. Azizkhan-Clifford, Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer, J. Cell. Physiol. 188 (2001) 143–160.
[18] C. Gooding, F. Clark, M.C. Wollerton, S.N. Grellscheid, H. Groom, C.W. Smith, A class of human exons with predicted distant branch points revealed by analysis of AG dinucleotide exclusion zones, Genome Biol. 7 (2006) R1.
[19] C.W. Smith, T.T. Chu, B. Nadal-Ginard, Scanning and competition between AGs are involved in 3′ splice site selection in mammalian introns, Mol. Cell. Biol. 13 (1993) 4939–4952.
[20] G. Svineng, R. Fässler, S. Johansson, Identification of beta1C-2, a novel variant of the integrin beta1 subunit generated by utilization of an alternative splice acceptor site in exon C, Biochem. J. 330 (1998) 1255–1263.
[21] S. Iwahori, Y. Yasui, A. Kudoh, Y. Sato, S. Nakayama, T. Murata, H. Isomura, T. Tsurumi, Identification of phosphorylation sites on transcription factor Sp1 in response to DNA damage and its accumulation at damaged sites, Cell. Signal. 20 (2008) 1795–1803.
[22] P. Fojas de Borja, N. Collins, P. Du, J. Azizkhan-Clifford, M. Mudryj, Cyclin A-CDK phosphorylates Sp1 and enhances Sp1-mediated transcription, EMBO J. 20 (2001) 5737–5747.
[23] X.R. Mao, A.M. Moerman-Herzog, Y. Chen, S.W. Barger, Unique aspects of transcriptional regulation in neurons – nuances in NFkappaB and Sp1-related factors, J. Neuroinflammation 6 (2009) 16.
[24] J. Lee, D. Suh, J. Kang, M. Kim, H. Park, M. Lee, J. Kim, B. Jeon, H. Roh, M. Yu, K. Choi, K. Kim, M. Hur, Transcriptional activity of Sp1 is regulated by molecular interactions between the zinc finger DNA binding domain and the inhibitory domain with corepressors, and this interaction is modulated by MEK, J. Biol. Chem. 280 (2005) 28061–28071.