Hsp90-binding immunophilin FKBP51 forms complexes with hTERT enhancing telomerase activity ☆

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ABSTRACT

FK506-binding proteins are members of the immunophilin family of proteins. Those immunophilins associated to the 90-kDa-heat-shock protein, Hsp90, have been proposed as potential modulators of signalling cascade factors chaperoned by Hsp90. FKBP51 and FKBP52 are the best characterized Hsp90-bound immunophilins first described associated to steroid-receptors. The reverse transcriptase subunit of telomerase, hTERT, is also an Hsp90 client-protein and is highly expressed in cancer cells, where it is required to compensate the loss of telomeric DNA after each successive cell division. Because FKBP51 is also a highly expressed protein in cancer tissues, we analyzed its potential association with hTERT·Hsp90 complexes and its possible biological role. In this study it is demonstrated that both immunophilins, FKBP51 and FKBP52, co-immunoprecipitate with hTERT. The Hsp90 inhibitor radicicol disrupts the heterocomplex and favors the partial cytoplasmic relocalization of hTERT in similar manner as the overexpression of the TPR-domain peptide of the immunophilin. While confocal microscopy images show that FKBP51 is primarily localized in mitochondria and hTERT is totally nuclear, upon the onset of oxidative stress, FKBP51 (but not FKBP52) becomes mostly nuclear colocalizing with hTERT, and longer exposure times to peroxide favors hTERT export to mitochondria. Importantly, telomerase activity of hTERT is significantly enhanced by FKBP51. These observations support the emerging role assigned to FKBP51 as antiapoptotic factor in cancer

development and progression, and describe for the first time the potential role of this immunophilin favoring the clonal expansion by enhancing telomerase activity.

1. Introduction

Immunophilins belong to a family of proteins that exhibit high specificity in binding immunosuppressive agents. The signature domain of this family is the PPIase (peptidyl-prolyl-(cis/trans)-isomerase) domain (Erlejman et al., 2013; Guy et al., 2015; Storer et al., 2011), where immunosuppressive drugs bind and inhibit the intrinsic PPIase activity of these proteins. Immunophilins are classified as FKBPs (or FK506-binding proteins) when they bind the macrolide FK506, and CyPs (or cyclophilins) when they bind the cyclic undecapeptide cyclosporine A (Erlejman et al., 2014b; Ratajczak et al., 2003). The low molecular weight immunophilins FKBP12 (12-kDa) and CyPA (17-kDa) are responsible for the immunosuppressive action by inhibition of the Ser/Thr-phosphatase activity of PP2B/calcineurin in lymphocytes (Ho et al., 1996). Although early studies suggested that FKBP51 could be related to immunosuppression via calcineurin inhibition (Baughman et al., 1995; Li et al., 2002; Weiwad et al., 2006), other works have stated that FKBP51 lacks this action (Stechschulte and Sanchez, 2011; Xu et al., 2002) or have postulated that the mechanism could be different (Kim et al., 2012). In addition to the PPIase domain, high molecular weight immunophilins show other domains such as the tetratricopeptide-repeat motif (TPR), through which they bind to Hsp90 (Storer et al., 2011). The biological roles of these proteins are still under investigation and are not entirely elucidated at the present time.

FKBP51 is a 51-kDa TPR-domain protein that was first described associated to steroid-receptors along with Hsp90, Hsp70 and p23 (Nair et al., 1997). FKBP51 shares 75% of similarity with FKBP52, a 52-kDa immunophilin able to interact with the dynein/dynactin motor complex favoring the retrotransport of soluble proteins (Guy et al., 2015; Lagadari et al., 2015; Salatino et al., 2006; Storer et al., 2011). FKBP52 also plays a role during the nuclear import mechanism of steroid receptors through the nuclear pore (Echeverria et al., 2009; Galigniana et al., 2010a). On the other hand, FKBP51 shows negligible affinity for dynein (Wochnik et al., 2005), is not recovered in steroid receptor complexes in the nucleoplasm during the early steps of steroid receptor nuclear localization as FKBP52 is (Galigniana et al., 2010b), and the high expression of FKBP51 favors the nuclear exclusion of interacting transcription factors (Banerjee et al., 2008; Erlejman et al., 2014a; Galigniana et al., 2010b). Recently, we have demonstrated that FKBP51 is a novel mitochondrial factor (Gallo et al., 2011).

Several evidences suggest that FKBP51 acquires a pro-oncogenic potential when its expression is deregulated (Mazaira et al., 2016; Romano et al., 2010b). Thus, FKBP51 positively regulates melanoma stemness and metastatic potential (Romano et al., 2013). FKBP51 is thought to be a key factor in the progression and chemotherapeutic response of pancreatic adenocarcinoma (Ellsworth et al., 2013), and it is close-related to acute lymphoblastic leukemia and several variants of breast, ovary and lung tumor pathologies (Romano et al., 2010b).

Cancer cells are also characterized by possessing high telomerase activity, which is essential for their rapid clonal expansion (Eisenstein, 2011). Telomerase is a ribonucleoprotein that compensates for the loss of telomeric DNA by adding repeated sequences to the chromosome ends using its intrinsic RNA component as a template for DNA synthesis. The reverse transcriptase subunit of telomerase, hTERT, contains the catalytic activity of the enzyme, whereas the associated RNA component, hTR, serves as the template for synthesis of telomeric sequences. Both subunits are essential for restoring telomerase activity in vitro and the introduction of these genes into normal cells extend the life span of these otherwise mortal cells (Feng et al., 1995; Meyerson et al., 1997). It has been demonstrated that the Hsp90 chaperone complex is required for assembly of telomerase (Holt et al., 1999). The minimal components necessary for active telomerase assembly are hTERT, hTR, Hsp90, p23, Hsp70, Hop/p60, and Hsp40. Hsp90 and p23 associate in the absence of hTR and remain associated with the active telomerase, whereas Hsp70 is only bound to inactive forms (Forsythe et al., 2001). Interestingly, Hop/p60 is an Hsp90-binding TPR-domain protein also required for steroid-receptor assembly, although it is not part of the heterocomplex associated to mature receptors, where it is replaced by an immunophilin (Galigniana et al., 2010a). In other words, the assembly complex of hTERT shows similar composition and features as those described for steroid receptors.

The Hsp90 network facilitates the effective operation of the telomere system (DeZwaan and Freeman, 2010), including its cell cycle-dependent intranuclear localization. Nonetheless, the complexity level for hTERT regulation is unexpected when compared with other cellular polymerases (Hukezalie and Wong, 2013), such that it has been postulated the existence of still unrevealed factors able to create dynamic telomere environments (DeZwaan and Freeman, 2010). Inasmuch as telomerase activity is significantly increased in those cell types where FKBP51 is also highly expressed, and because both proteins are Hsp90-interacting proteins, in this study we explored whether this TPR-domain immunophilin forms complexes with hTERT and its potential role in the regulation of telomerase activity.

2. Materials and methods

2.1. Materials

Radicicol and DMSO were from Sigma (St. Louis, MO). FK506 was from LC Laboratories (Wobrun, MA). Hydrogen peroxide was from Merck Argentina. Rabbit monoclonal IgG anti-FKBP51 was from Affinity BioReagents (Golden, CO). The MG19 mouse monoclonal IgG anti-FKBP51 was produced in the laboratory (Quintá et al., 2010). UP30 rabbit antiserum against FKBP52 was a generous gift by Dr. William Pratt (University of Michigan). The AC88 mouse monoclonal IgG against Hsp90 was from StressMarq Biosciences (Victoria, Canada). Anti-HA mouse monoclonal IgG, rabbit polyclonal IgG anti-hTERT, rabbit polyclonal IgG anti-Tom-20, and goat polyclonal IgG anti-lamin B were from Santa Cruz Biotech (Santa Cruz, CA). Mouse IgG against actin and mouse IgG against Cox-IV were from Abcam (Cambridge, UK). Mouse IgG against the flag peptide, and HRP-conjugated Protein-A were from Sigma. All Alexa-labelled secondary antibodies and H2DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate) were from Molecular Probes (Eugene, OR, USA). The siRNA for FKBP51 and control siRNA were from Thermo Scientific Dharmacon (Chicago, IL, USA).

2.2. Cell treatments

All cell lines were from ATCC, except the 293-51 + cell line that constitutively overexpresses hFKBP51, which was developed in our laboratory as described in a previous work (Erlejman et al., 2014a). The natural control used for 293-51 + cells was the 293 cell line from which the former derives. L1-3T3 fibroblasts were selected due to the good visualization of mitochondria by indirect immunofluorescence. Cervix adenocarcinoma HeLa cells were chosen due to the high endogenous expression level of both hTERT and FKBP51. 293T cells were used for some experiments where overexpression of proteins was required to ensure high transfection efficiency. All cell types were grown in high glucose DMEM (Thermo Fisher Sci, Waltham, MA) supplemented with 10% calf bovine serum (Internegocios, Mercedes, Argentina), 2 mM glutamine, 50 U/ml penicillin and 50 mg/ml streptomycin (all of them from Sigma). Transfections were performed when cells reached 40−50% confluence using the TransFast reagent (Promega, Madison,WI). After 24 h post-transfection, cells were treated for 16 h with 0.5 mM H_2O_2 and cell death was measured by double-counting of viable cells by trypan blue exclusion in a Neubaüer camera, and by spectrometry at 570 nm after staining cells with 0.5% crystal violet (Colo et al., 2008). Subcellular fractionation into nuclei, cytosol and mitochondria was achieved exactly as it was described in a previous study (Gallo et al., 2011). NIH-3T3 fibroblasts were transformed into a tumorigenic cell line by stable transfection of the v-Ha-Ras oncogene generously gifted by Dr. Elisa Bal de Kier Joffe (Aguirre-Ghiso et al., 1999). The knock-down of FKBP51 expression by specific siRNA was achieved as described in previous studies (Quinta and Galigniana, 2012; Quintá et al., 2010).

2.3. hTERT immunoprecipitation

When the culture reached ∼70% of confluence, cells were harvested by trypsinization, washed with saline-phosphate buffer, and homogenized in one volume of lysis buffer (0.01%NP-40, 10 mM Tris at pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 20% glycerol and 20 mM Na_2MoO_4) supplemented with 1 µg/µl RNAsa and 1 µg/µl DNAsa) as described (Galigniana, 1998). After 20 min on ice, a 10-sec pulse sonication at 20 W was performed followed by a centrifugation at 13,000 rpm for 15 min at 4 °C. The supernatants (250 µl) were rotated for 2.5 h at 4 °C with 75 µl of Protein G-Sepharose (50% w/v) and 1 µg of anti-hTERT IgG. Pellets were washed four times with 1 ml of lysis buffer and proteins were resolved by Western blot.

2.4. Indirect immunofluorescence assays

Cells were grown on collagenized coverslips, fixed with methanol at −20 °C for 10 min, and incubated overnight at 4 °C with 1/100 dilution of primary antibody and 1 h at room temperature with 1/200 dilution of secondary antibody. Coverslips were mounted in a glycerol based media with an anti-fade solution. Confocal microscopy images were acquired with a Nikon Eclipse-E800 confocal microscope using a Nikon DS-U1 camera with ACT-2U software. Co-localization analyses were performed using the co-localization plug-in of the Fiji program (v.1.45) (NIH; Bethesda, MA, USA), which uses a range of algorithms such as co-localization thresholds, Pearson's linear correlation coefficient, overlap and Manders coefficients (Manders et al., 1993). We collected confocal z-series of cells (40 optical slices, airy unit = 1 airydisc at 0.25 mm intervals using 63× objective), and then images were deconvolved using Huygens compute engine 3.5.2p3 64b (closed platform). Finally, images were imported to the Fiji program to determine the degree of overlapping.

2.5. Telomerase enzymatic activity

Telomerase activity was measured using a standard commercial kit (TeloTAGGG-Telomerase PCR ELISA-Plus, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

3. Results

3.1. FKBP51 favors cell survival and is overexpressed in cancer cells

Confocal microscopy images show that FKBP51 co-localizes with the mitochondrial marker MitoTracker (Figure 1A). This confirms our previous observations where the immunophilin was reported as a novel mitochondrial factor (Gallo et al., 2011). Cell treatment with 0.5 mM H_2O_2 for 1 h favors the rapid (20−30 min) nuclear relocalization of FKBP51 (Figure 1B). The mitochondrial-nuclear trafficking observed by indirect immunofluorescence can also be evidenced by conventional biochemical fractionation (Figure 1C). This property is not seen for FKBP52, which shows the same cytoplasmic and
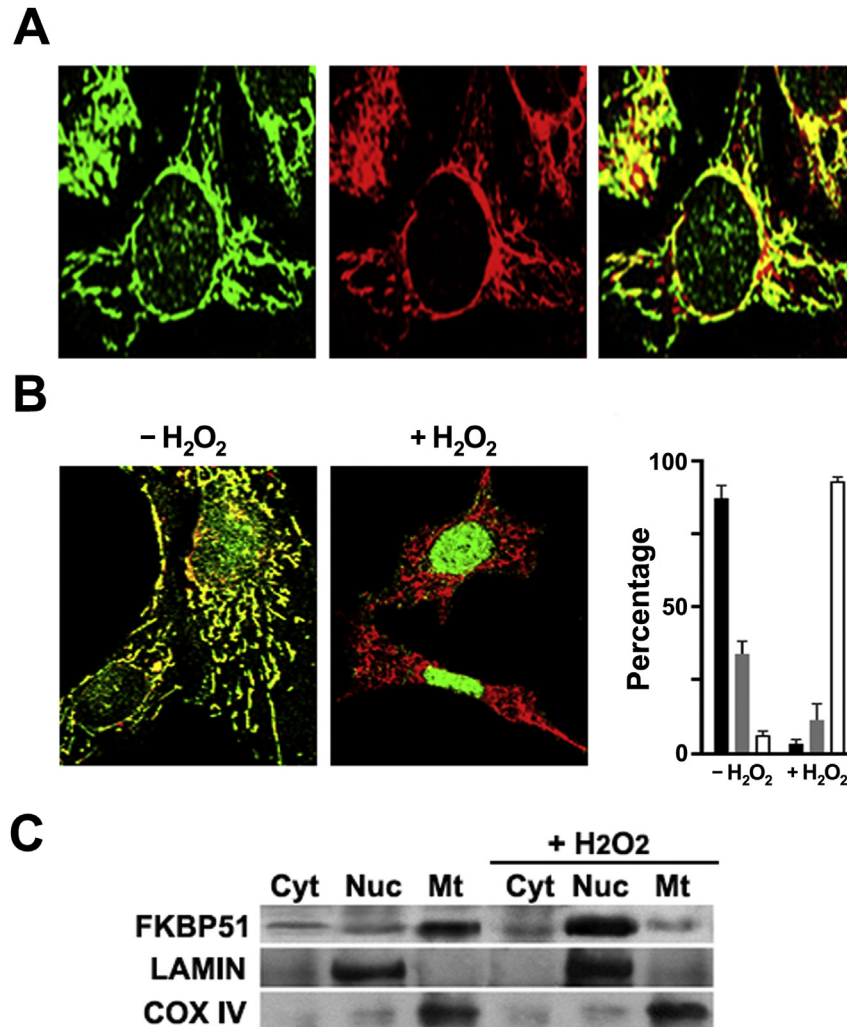
Figure 1 − FKBP51 is a mitochondrial protein that migrates to the nucleus under oxidative stress conditions. (A) FKBP51 (green) is visualized in 3T3-L1 fibroblasts by confocal microscopy colocalizing with MitoTracker (red). (B) FKBP51 concentrates in nuclei in cells treated with 0.5 mM H_2O_2 for 1 h. The bar graph (mean ± SEM, n = 4) depicts the percentage of cells showing mitochondrial pattern (*black*), partial nuclear staining (*gray*) and full nuclear accumulation of FKBP51 (*white*) in both conditions. (C) 3T3-L1 cells were treated with 0.5 mM H_2O_2 and fractioned into cytosolic (*Cyt*), nuclear (*Nuc*) and mitochondrial (*Mt*) fractions. Lamin B and COX-IV were used as nuclear and mitochondrial markers.

partially nuclear localization with and without H_2O_2 (Supplementary Figure S-1).

Oxidative stress is a well known condition associated to cancer metabolism, such that distorted redox-dependent signaling pathways orchestrate anomalous events that result in cell resistance to stress and apoptosis, as well as aberrant proliferation and efficient repair mechanisms (Manda et al., 2015; Thorne and Campbell, 2015). The expression of FKBP51 appears to be higher in cancer cell lines than normal cells (Figure 2A). To provide more direct evidence, normal NIH-3T3 fibroblasts were transformed into tumorigenic cells by v-Ras oncogene (Aguirre-Ghiso et al., 1999). Cell phenotypes are shown in Figure 2B and the induction of FKBP51 expression in cells with identical genetic background is clearly observed in the Western blot. This agrees with those differences evidenced in Figure 2A for FKBP51 expression, which

results of the malignant nature of cancer cells compared to normal cells.

Importantly, the overexpression of the immunophilin is associated to resistance of the cells to death by oxidative injury, whereas its knock-down significantly sensitize cells to lower concentrations of peroxide (Figure 2C). This effect of FKBP51 confirms the antiapoptotic action we suggested in a previous study (Gallo et al., 2011) and is exclusive for FKBP51 since the overexpression of FKBP52 shows no significant action (data not shown) regarding the control transfected with empty vector. Control assays performed with cells treated with 10 μM H2DCF-DA, a cell-permeant fluorescent free radical sensor, showed the expected more intense signal of reactive-oxygen species in cancer cells than normal cells (Supplementary Figure S-2), as well as a greater pool of nuclear FKBP51 (Supplementary Figure S-3).
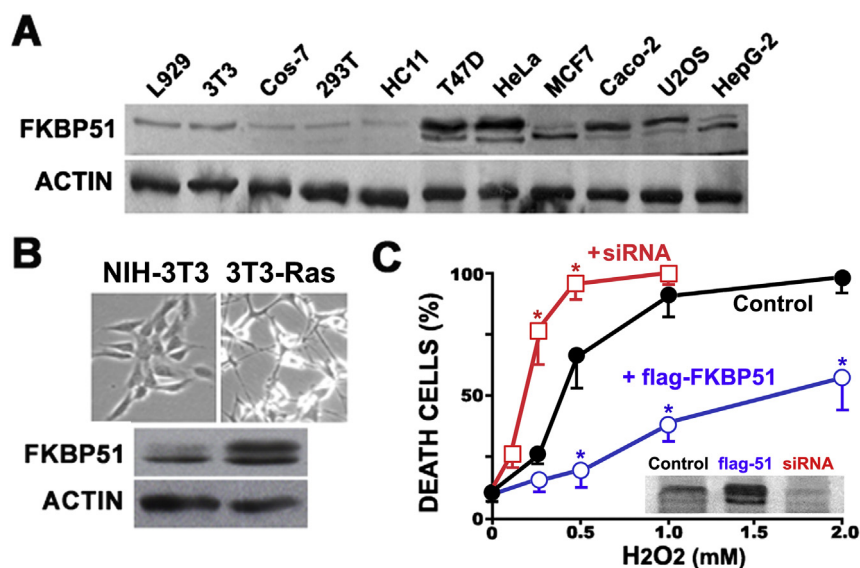
Figure 2 − FKBP51 is overexpressed in cancer cells. (A) Cell lysates (30 μg of protein) from normal cell lines (L929, NIH-3T3, Cos-7, 293T and HC11) and cancer cell lines (T47D, HeLa, MCF7, Caco-2, U2OS and HepG-2) were Western blotted for FKBP51 and actin (loading control). (B) FKBP51 becomes overexpressed when NIH-3T3 cells acquire malignant phenotype by *v*-Ha-Ras oncogene expression. (C) 293T cells were transfected with pCI-Neo-*flag*-hFKBP51 (blue), siRNA for FKBP51 (red) or irrelevant nucleic acid (black). After 36 h, cells were treated overnight with 0.5 mM H_2O_2 and cell viability was quantified (mean ± SEM, n = 3). *p < 0.01. The insert shows a Western blot for FKBP51 in each condition.

3.2. FKBP51 co-localizes with hTERT

Inasmuch as cancer cells exhibit high level of expression of telomerase activity and its catalytic subunit hTERT is a known Hsp90-client-protein (Forsythe et al., 2001), we explored the possibility that the Hsp90-binding immunophilin FKBP51 could be a novel member of the hTERT·Hsp90 heterocomplex. Figure 3A shows confocal microscopy images for endogenous FKBP51 (green) (in web version) and endogenous hTERT (red) (in web version) in HeLa cells. In basal conditions, hTERT is primarily nuclear, whereas FKBP51 is ubiquitously distributed in interphase cells (upper row), although it is mainly located in the cytoplasm rather than in nuclei. As the mask image demonstrates (extreme right image), the nuclear fraction of FKBP51 colocalizes with hTERT. This co-localization was also observed in mitotic HeLa cells (middle row). Treatment with H_2O_2 triggered the nuclear translocation of FKBP51, which increased its nuclear co-localization with hTERT (lower row). The profiles of intensity of fluorescence shown in Figure 3B for both proteins confirm their nuclear co-localization.

To establish the significance of the true co-localization observed by microscopy, we determined the Pearson and Mander's coefficient (Manders et al., 1993) to measure the degree of correlative variation of FKBP51 and hTERT. This analysis yielded values closer to one when the two signals change together. As is shown in the representative data of Figure 3C, the R colocalization coefficient was ∼0.8 for each cell analyzed. In addition, a quantitative correlation analysis using Mander's algorithm showed that the co-localization rate in the nucleus was ∼50%. Interestingly, a fraction of hTERT is relocated to the cytoplasm in H_2O_2-treated cells, although the cytoplasmic fractions of both proteins exhibit

negligible true co-localization suggesting that this pool of hTERT is not significantly associated to FKBP51.

3.3. FKBP51 forms complexes with hTERT enhancing telomerase enzymatic activity

Because true co-localization does not univocally means protein−protein interaction, those analyses discussed above were corroborated by direct co-immunoprecipitation assay of endogenous FKBP51 with endogenous hTERT (Figure 4A). FKBP52, a highly homologous partner of FKBP51, was also recovered in the complex when hTERT was immunoprecipitated. FKBP52 has also been found associated with other Hsp90 client-proteins where it favors their rapid and efficient retrotransport rate on microtubules tracks thanks to its capacity to interact via its PPIase domain with the dynein/dynactin motor complex, although FKBP52 is not absolutely required for the late nuclear accumulation of these factors since slow trafficking takes place anyway (perhaps due to simple diffusion) (Galigniana, 2012; Galigniana et al., 2010a, 2010b; Guy et al., 2015; Storer et al., 2011; Wochnik et al., 2005). Figure 4B shows the reverse co-immunoprecipitation of hTERT with FKBP51, a complex that is not disturbed by the macrolide FK506.

Next, we asked whether these immunophilins influence telomerase enzymatic activity. Because FKBP51 is overexpressed in tumor cells and show antiapoptotic properties, a positive action on telomerase activity was expected. Figure 4C confirms such *a priori* prediction. The 293-51 + cell line stably transfected with hFKBP51 shows higher enzymatic activity compared to the 293 parental cell line (*blue bars*) (in web version). Similar results were obtained with 293T
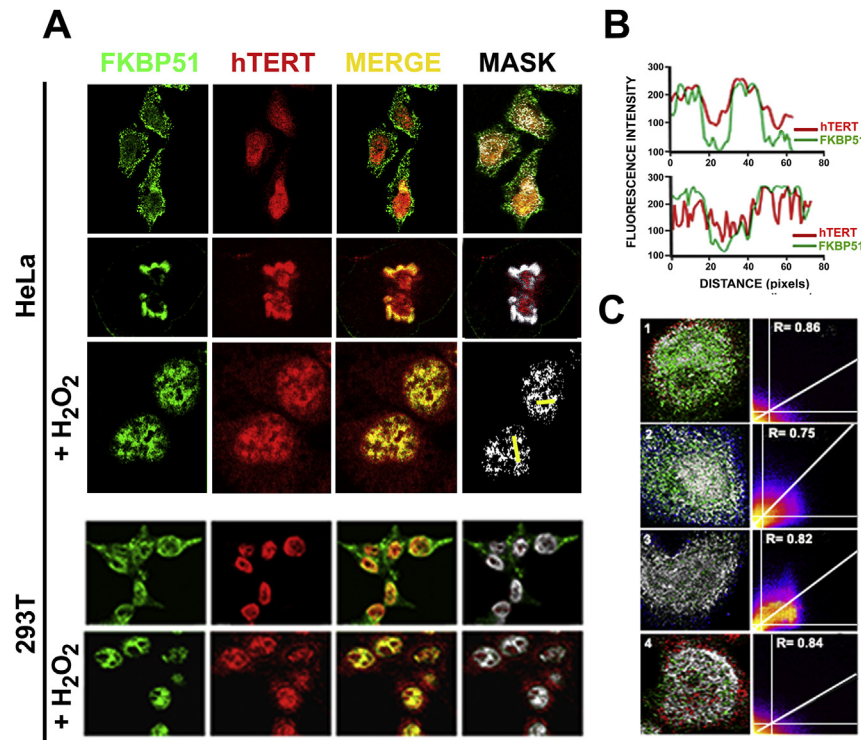
Figure 3 − FKBP51 co-localizes with hTERT. (A) Colocalization of FKBP51 (*green*) and hTERT (*red*) by confocal microscopy in HeLa cells during interphase (*upper line*), mitosis (*middle line*), and peroxide-induced nuclear accumulation of the immunophilin (*lower line*). Similar patterns were observed in 293T cells. (B) Intensity of fluorescence profiles of FKBP51 and hTERT for the yellow scan depicted in the nuclei of HeLa cells shown in the lower right corner. (C) True co-localization analysis for non-stimulated 293T cells (basal condition). Scatterplots shown an R colocalization values equal to or around 0.8, supporting the same subcellular distribution of both proteins.

fibroblasts (*black bars*) (in web version) and HeLa cells (*brown bars*) (in web version) transiently transfected with FKBP51 when they were compared to the basal activity of cells transfected with empty vector. Interestingly, FKBP52 also shows equivalent stimulant action suggesting redundancy of action on hTERT activity. To assess the requirement of the FKBP51·Hsp90 association, a TPR-domain mutant (K352A) of FKBP51 was overexpressed in 293T cells. As it was expected, the lack of an efficient interaction with Hsp90 decreased telomerase activity. This was also seen in HeLa cells when the Hsp90 inhibitor radicicol was assayed. Interestingly, even though the macrolide FK506 has no effect on the protein−protein association between hTERT and FKBP51 (Figure 4B), it does prevent the stimulant action of the immunophilin on telomerase activity of HeLa cells (Figure 4C), suggesting that the PPIase enzymatic activity of FKBP51 is actually involved in the regulation of telomerase activity, which may have pharmacological relevance. That interpretation was confirmed by transfection of a mutant of FKBP51 in the PPIase site. The lack of enzymatic activity abolishes the stimulant effect of FKBP51 overexpression.

3.4.   *Hsp90-binding favors hTERT nuclear localization*

Because the Hsp90 inhibitor radicicol abolishes the stimulant action of FKBP51 on telomerase activity (Figure 4C), the composition of endogenous hTERT heterocomplex was analyzed in radicicol-treated HeLa cells by co-immunoprecipitation assay (Figure 5A). Both Hsp90 and its co-chaperone FKBP51 were dissociated from hTERT in radicicol-treated cells, demonstrating the disruption of the complexes in the presence of the Hsp90 inhibitor. Indirect immunofluorescence images show endogenous hTERT in the cytoplasm rather than in nuclei, an effect that parallels that also observed under similar conditions with steroid receptors since the retrotransport is entirely dependent on Hsp90 complexes (Galigniana et al., 2010b). Figure 5B shows that when cells are exposed to radicicol, hTERT becomes cytoplasmic whereas FKBP51 fully accumulates in the nuclei.

The overall behavior of the hTERT·Hsp90 complex resembles that we have previously observed for steroid receptors. In those studies, it was reported that the TPR-domain of immunophilins plays an active role in the subcellular distribution of Hsp90 client-factors, such that its overexpression delocalizes the receptor from nuclei (Galigniana et al., 2010b). Therefore, to evaluate this property, a flag-tagged TPR peptide was overexpressed and then, the subcellular distribution of hTERT was analyzed by confocal microscopy. Figure 5C shows that in those cells overexpressing the TPR peptide (green) (in web version), hTERT (red) (in web version) is delocalized and becomes more cytoplasmic. Notably, those two cells in the same field that were not co-transfected and are pointed by a
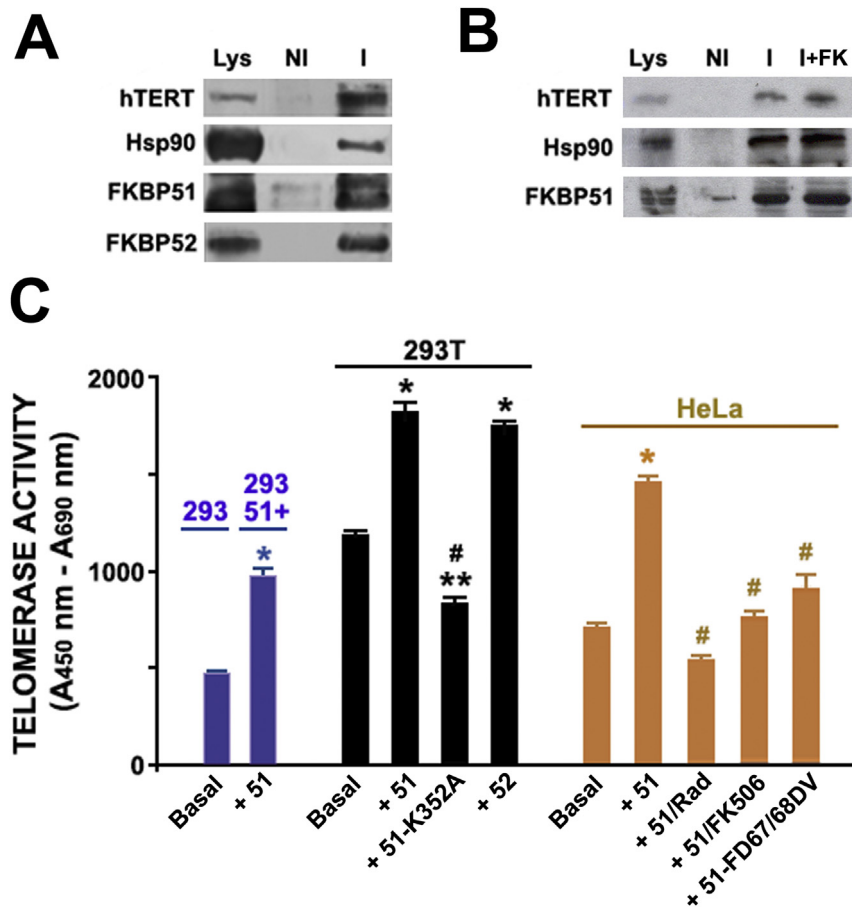
Figure 4 − FKBP51 forms heterocomplexes with hTERT enhancing telomerase activity. (A) hTERT was immunoprecipitated from whole HeLa cells lysates (*Lys*) and proteins were resolved by Western blot. NI = Non-immune IgG; I = Anti-hTERT IgG. (B) Telomerase activity was measured in: *Blue*) 293 cells stably transfected with FKBP51 (clone 293-51+); 293 wild type cells were used as control. *Black*) 293T fibroblasts transfected with empty vector (*Basal*), pCI-Neo-hFKBP52 (+*52*), pCI-Neo-flag-hFKBP51 (+*51*),or pCI-Neo-flag-hFKBP51-K352A (+*51 K352A*), a point mutant in the TPR domain that is unable to bind Hsp90. *Brown*) HeLa cells transfected with vector (*Basal*), pCI-Neo-flag-hFKBP51 (+*51*) or pCI-Neo-hFKBP51-FD67/68DV (a mutant in the PPIase domain unable to show enzymatic activity). Those FKBP51 transfected cells were pretreated or not for 1 h with 1 µM radicicol (+*51/Rad*) or 1 µM tacrolimus (+*51/FK506*). Bars represent the mean ± SEM (n = 5). Significantly different from basal condition at * p < 0.01 or **p < 0.05. Significantly different from +51 at #p < 0.01.

white arrow, i.e., cells transfected with hTERT (red) (in web version), but not with TPR peptide (green) (in web version), show nuclear fluorescence only. In summary, the association of hTERT with Hsp90-based heterocomplex appears to determine the nuclear accumulation of hTERT.

3.5.   Cytoplasmic hTERT is targeted to mitochondria or proteasome degradation

While an important relocalization of FKBP51 from mitochondria to the nucleus occurs rapidly (~30 min) (Gallo et al., 2011), the full nuclear export of hTERT in the presence of radicicol requires ~4 h (Figure 5). In order to determine whether both events are always related, HeLa cells were treated with H_2O_2, other known condition where FKBP51 migrates from mitochondria to nuclei (Figure 1). Figure 6A shows that after 1 h treatment, FKBP51 (red) is already nuclear, and after 4 h with H_2O_2, hTERT became cytoplasmic.

The subcellular fractionation shown at the bottom of panel 6A demonstrates that under the latter condition, most of the cytoplasmic pool of hTERT is mitochondrial. This is in agreement with the observed relocalization of hTERT by oxidative stress, where it is supposed to perform non-canonical and telomere-independent functions such as enhancing the resistance to apoptosis (Maida and Masutomi, 2015; Massard et al., 2006).

To determine whether or not this trafficking is reversible, cells were exposed to H_2O_2 for up to 3 h, and the medium was replaced by regular peroxide-free medium. Cells were incubated for an additional hour. Figure 6B shows that while the nuclear pool of FKBP51 (green) (in web version) is relocated to mitochondria, hTERT (red) (in web version) remains cytoplasmic. In summary, this experiment shows that both treatments, Hsp90 inhibition and oxidative stress, promote similar relocalization of both proteins, an effect that was not observed for FKBP52 in peroxide-treated cells (Figure S-1).
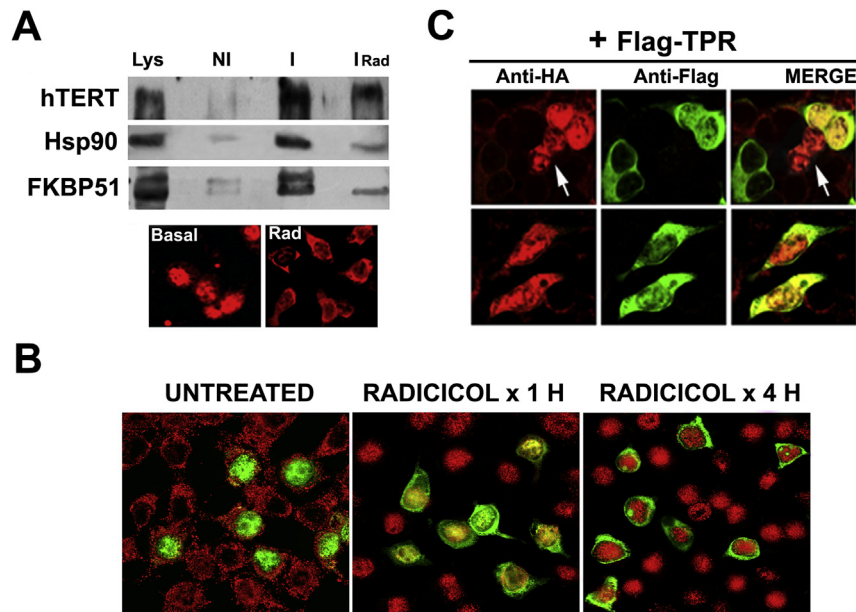
Figure 5 – **The disruption of hTERT•Hsp90 assembly favors hTERT nuclear export. (A)** HeLa cells were pretreated for 4 h with 1 μM radicicol (*Rad*), hTERT was immunoprecipitated (*I* and *I_{Rad}*), and proteins were resolved by Western blot. *NI*: non-immune IgG, *Lys*: whole lysate. The lower panel shows the subcellular redistribution of endogenous hTERT (*red*) for each condition. **(B)** 293T Cells transfected with pCI-Neo-hEST2-HA encoding for the human telomerase catalytic subunit were incubated for 1 h or 4 h with 1 μM Rad, fixed, and subjected to an indirect immunofluorescence for both endogenous FKBP51 (*red*) and expressed HA-hTERT (green). Note the cytoplasmic redistribution of HA-hTERT and the nuclear accumulation of FKBP51 in the presence of the Hsp90-disrupting agent. **(C)** 293T Cells were co-transfected with pCMV6-flag-TPR and pCI-Neo-hEST2-HA, and the cytoplasmic relocalization of HA-hTERT (*red*) was evaluated in flag-positive cells (*green*). The white arrow shows two cells transfected with HA-hTERT only. Note the nuclear fluorescence compared to TPR co-transfected cells.

Time-lapsed studies demonstrated that the utmost amount of exported hTERT in the presence of 0.5 mM H_2O_2 is reached after 4 h. Therefore, a cell fractionation was performed to determine the expression level of both FKBP51 and hTERT under such condition. As it was expected for any heat-shock protein, the expression level of FKBP51 was rapidly induced by H_2O_2 and the immunophilin was concentrated in the nucleus. This is evidenced in Figure 6C after 1 h treatment and, at a greater extension, after 4 h with peroxide. Surprisingly, hTERT expression is also induced by oxidative stress. On the other hand, radicicol promotes hTERT degradation by the proteasome, as it is judged by the protective action of the proteasome inhibitor MG132. In other words, if hTERT is exported from nuclei and is not protected in mitochondria, it tends to be degraded by proteasomal degradation.

4. Discussion

This study demonstrates the association of the Hsp90-binding immunophilin FKBP51 with hTERT. Therefore, it can be postulated that FKBP51 is one of the still unrevealed factors whose existence was recently predicted to allow the creation of dynamic telomere environments (DeZwaan and Freeman, 2010). The association of this TPR-domain factor with hTERT enhances telomerase activity, an observation that is in line with the emerging actions of FKBP51 in tumor cells, cancer development, and progression (Mazaira et al., 2016; Ratajczak et al., 2015; Romano et al., 2010b). Such stimulant action of FKBP51 on hTERT activity is in agreement with other features of this immunophilin such as its antiapoptotic action, its overexpression in tumor cells, its strong nuclear colocalization with hTERT, and the absolute requirement of hTERT enzymatic activity to prevent the incremental shortening of telomeres allowing cells to circumvent the Hayflick limit of cell divisions before becoming senescent (Holliday, 1996). The stimulant action of FKBP51 is redundant with that observed for FKBP52 (Figure 4C), a partner protein that shows high homology (Storer et al., 2011). In contrast with FKBP51, indirect immunofluorescence confocal microscopy images showed no significant variation for the ubiquitous subcellular localization of FKBP52 in peroxide-treated cells (Supplementary Figure S-2). Therefore, in this study all our efforts were concentrated on FKBP51 properties.

The facts that FKBP51 is overexpressed in cancer cells (Figure 2A), its expression is increased when a normal cell is transformed into a cancer cell (Figure 2B), and also more concentrated in nuclei of cancer cells (Supplementary Figure S-3) make this immunophilin attractive as a potential pro-oncogenic factor. Interestingly, FKBP51 binding to Hsp90 favors the recruitment of the co-chaperone p23, which positively regulates androgen receptor signaling in prostate cancer (Ni et al., 2010), and is associated with chemoresistance and radioresistance (Pei et al., 2009; Romano et al., 2010a). It has
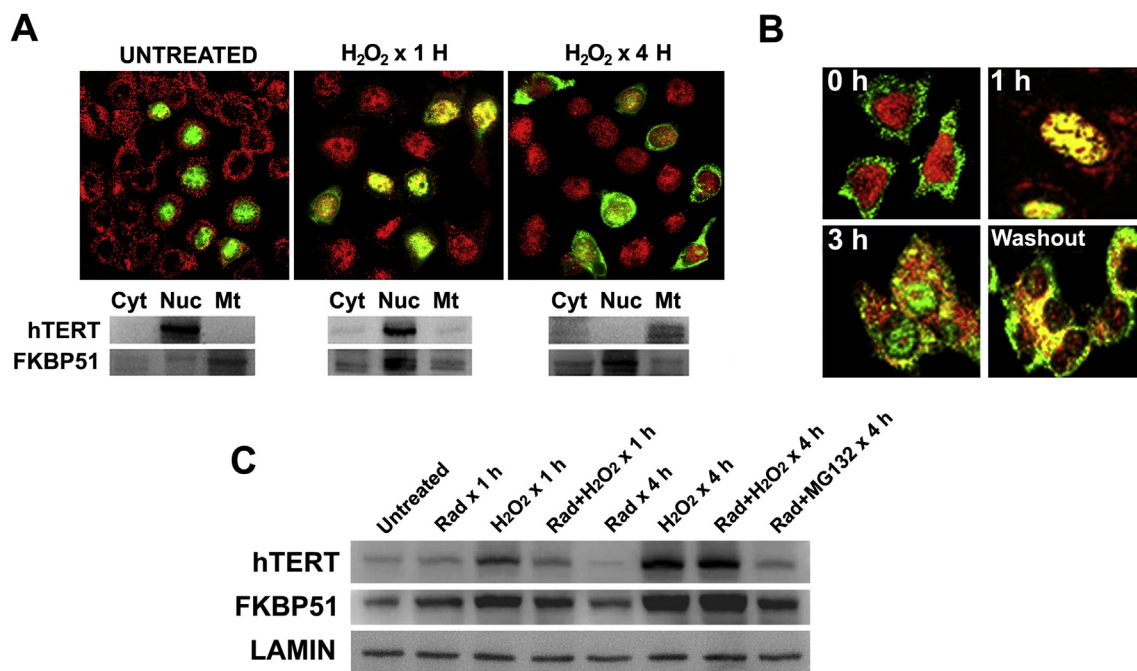
Figure 6 − Oxidative stress promotes hTERT nuclear export to mitochondria. (A) HeLa cells were transfected with pCI-Neo-hEST2-HA, treated with 0.5 mM H₂O₂ for 1 h or 4 h, and the subcellular localization of endogenous FKBP51 (*red*) and HA-hTERT (*green*) was visualized by confocal microscopy. Western blots show the localization of both proteins after a subcellular fractionation into cytosol (*Cyt*), nuclei (*Nuc*) and mitochondria (*Mt*). **(B)** HeLa cells were treated with 0.5 mM H₂O₂ for 1 h, 3 h, or 3 h followed by an extra 1 h incubation without peroxide (4 h total). The reversion of the subcellular redistribution of endogenous hTERT (*red*) and endogenous FKBP51 (*green*) was evaluated by confocal microscopy. **(C)** Cells were treated as described in the figure and 50 μg proteins were resolved by Western blot. Lamin B was used as loading control.

also been shown by siRNA interference studies that FKBP51 suppresses the proliferation of colorectal adenocarcinoma (Mukaide et al., 2008). The Hsp90·p23 chaperone complex also interacts with hTERT favoring a conformation that enables its nuclear translocation (Forsythe et al., 2001; Holt et al., 1999). hTERT contains a bipartite nuclear localization signal that is responsible for nuclear import (Chung et al., 2012), a mechanism that must be active due to the molecular weight of the complex. Similar to steroid receptors, hTERT could use the same molecular machinery to be transported to the nucleus, in which case the role of FKBP52 is essential because it is the immunophilin that link the cargo with molecular motors. FKBP51, in contrast, cannot interact with the transport machinery (Wochnik et al., 2005), but seems to be crucial for the nuclear activity of telomerase. Therefore, both immunophilins are essential for hTERT function.

Figure 7 depicts an integrated model for the mechanism of action we propose for hTERT. hTERT is assembled with the Hsp90-based complex (Holt et al., 1999) in similar manner as it has been described for steroid-receptors (Mazaira et al., 2014; Pratt et al., 2004). Thus, hTERT is folded in a heterocomplex that contains a dimer of Hsp90, Hsp70, p23 and FKBP52. The presence of FKBP52 (Figure 4A) implies a role of this immunophilin in hTERT retrotransport via dynein/dynactin, i.e., the same mechanism already established for steroid

receptors, ecdysone receptor, RAC-3, viral particles, etc (Guy et al., 2015; Sivils et al., 2011). Even though Hsp70 associates with hTERT, Lee et al. have demonstrated that it readily dissociates when telomerase is folded into its active form (Lee et al., 2010). In other systems such as receptors (Davies et al., 2002; Gallo et al., 2007) and NF-κB (Erlejman et al., 2014a), both immunophilins FKBP51 and FKBP52 are functionally exchanged. Therefore, it is possible that FKBP51 replaces FKBP52 in the nuclear compartment when the transport to the nucleus was completed. Moreover, the nuclear accumulation of FKBP51 observed in cancer cells and upon the onset of oxidative stress (also present in cancer cells) makes this exchange more feasible.

In the nucleus, the final heterocomplex is targeted to chromosomes where telomerase activity protects the shortening of chromosome ends, or it could be dissociated (by a still unknown stimulus). This property may be due to interactions with other nuclear factors or because FKBP51 itself undergoes post-translational modifications. In this sense, previous (Gallo et al., 2011) and current studies (N.Zgajnar and M.D.Galigniana, unpublished results) indicates that FKBP51 shows several phosphorylated isoforms that are favored by selective hormonal stimuli. This can be evidenced in this study in several Western blots where FKBP51 shows more than one band. This pattern is modified by preincubation with alkaline
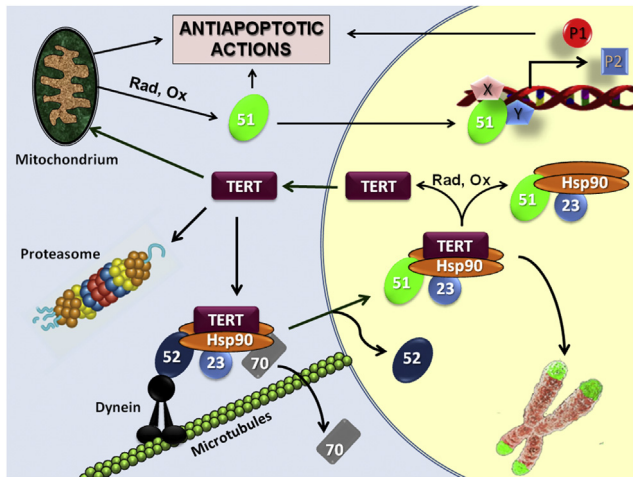
Figure 7 — Proposed model for the regulation of the hTERT•immunophilin interaction. hTERT is folded with the Hsp90-based heterocomplex and retrotransported by the FKBP52-motor protein complex. Active hTERT dissociates Hsp70, and FKBP52 may be exchanged by FKBP51, a situation that is favored in cancer cells and/or under stress conditions due to the high expression and nuclear accumulation of the immunophilin. hTERT is targeted to chromosomes where its telomerase activity prevents chromosome end shortening. Oxidative stress (Ox) or disruption of the Hsp90 complex, for example, in the presence of radicicol (Rad), favors hTERT nuclear export. This cytoplasmic pool can be imported by mitochondria or degraded by the proteasome. Both FKBP51 and mitochondrial hTERT play a protective role against apoptosis.

phosphatase to transform all isoforms into the one with greater electrophoretic mobility, as we have already reported in a previous study (Gallo et al., 2011).

While the chaperone complex is stable and could be recycled, naked hTERT is exported. This is supported by the fact that hTERT posses a nuclear export signal (Seimiya et al., 2000), and also because Hsp90-disrupting agents such as radicicol dissociates the complex (Figure 5A) making hTERT more cytoplasmic (Figure 5B), an effect also achieved when the TPR-peptide is overexpressed (Figure 5C). The latter condition also suggests a potential role of TPR-domain proteins for anchoring hTERT to the nuclear compartment, an observation postulated for steroid receptors (Galigniana et al., 2010b). Accordingly, the true co-localization analysis showed a negligible co-localization of this pool of hTERT with FKBP51. Cytoplasmic hTERT can be translocated into mitochondria enhancing the resistance to apoptosis, as it has already been suggested (Maida and Masutomi, 2015; Massard et al., 2006), whereas the soluble pool is targeted to proteasome degradation (Figure 6C). This property is frequent for most Hsp90-binding proteins when the chaperone is dissociated (Beliakoff and Whitesell, 2004).

Mitochondrial FKBP51 rapidly concentrates in the nucleus by oxidative stress, perhaps to enhance telomerase activity and, on the other hand, affecting the expression of key factors (generically named P1 and P2 in the model shown in Figure 7) that are required for cell survival and/or to prevent cell

senescence. Over time, hTERT is exported to mitochondria, an event perhaps facilitated by the chaperoning action of FKBP51 releasing hTERT from telomeric regions where it was anchored. Whatever the reason, this favors the activation of resistance mechanisms to apoptosis (Massard et al., 2006). Both proteins, hTERT and FKBP51, may act together in the same complexes, but also in a coordinated sequential manner at different periods of time since the nuclear translocation of FKBP51 is rapid (∼30 min) and the mitochondrial import of hTERT takes ∼4 h.

Is should be emphasized that FKBP52 appears to be redundant with FKBP51 to enhance telomerase activity. This argues in favor of a nuclear protein–protein mechanism of action for hTERT involving both immunophilins rather than to a direct mechanism associated to the subcellular relocalization of FKBP51. We speculate that the main functional difference between both immunophilins may be related to the retrotransport mechanism of hTERT since FKBP52·Hsp90 complexes (but not FKBP51·Hsp90 complexes) are able to associate efficiently to the dynein/dynactin motor complex favoring the nuclear accumulation of the cargo protein (Galigniana et al., 2010b; Wochnik et al., 2005). Of course, the overall induction of FKBP51 (rather than FKBP52) observed upon the onset of oxidative stress, a hallmark property shown by most cancer cells, ultimately leads to enhanced telomerase activity since the greater availability of an activator could play a role in the number of active hTERT oligomeres in the nucleus.

Interestingly, FKBP51 is concentrated in the periphery of the mitotic spindle still associated to hTERT. With this sole evidence, it is difficult to realize the significance of this specific arrangement of the protein during cell division. We speculate that, because FKBP51 associates to depolymerized tubulin (Chambraud et al., 2007), during cell division FKBP51 could be sequestered into these structures to avoid its interference in the formation of spindle fibers, whereas hTERT is simultaneously protected from degradation. Accordingly, it has been reported that in neurons, FKBP51 regulates clearance of microtubule-associated protein Tau and thus, it stabilizes microtubules (Jinwal et al., 2010). Clearly, microtubule stabilization should not take place during cell division. Nonetheless, this hypothesis should be demonstrated.

FKBP51 is an immunophilin that has been progressively associated to a role in cancer biology and the regulation of several signalling cascades. Even though FKBP51 properties are still far from being fully elucidated, it results clear that FKBP51 plays key roles in cell growth, proliferation and differentiation (Liu et al., 2007; Menicanin et al., 2009; Periyasamy et al., 2010; Quintá et al., 2010; Romano et al., 2011b; Toneatto et al., 2013). Thus, in addition to its known role on steroid receptor regulation (Erlejman et al., 2014b; Storer et al., 2011), it has also been demonstrated that in cases of idiopatic myelofibrosis, FKBP51 favors cell survival mechanisms by reducing the dephosphorylation rate of STAT5 (Komura et al., 2003). Moreover, FKBP51 controls NF-κB activation (Erlejman et al., 2014a; Romano et al., 2011a; Romano et al., 2015), the Akt-p38 pathway (Stechschulte et al., 2014), and regulates TNFα signalling cascade by forming complexes with TRAF2 (Romano et al., 2015), a known mediator of cell survival processes, proinflammatory action, and cancer progression via canonical NF-κB, JNK and p38 MAP kinase signalling

(Borghi et al., 2016). Recently, it has been evidenced a role of FKBP51 in autophagy as a prerequisite for antidepressant effects (Gassen et al., 2014, 2015b). FKBP51 associates with BECN1, changes its phosphorylation and protein levels, and enhances markers of autophagy and autophagic flux. The effects of antidepressants on autophagy as well as their physiological effects in mice and humans also depend on FKBP51. It has also been shown that FKBP51 inhibits GSK3β and favors the effects of distinct psychotropics (Gassen et al., 2016) and prevents the biological activity of the DNA-methyltransferase DNMT1, which impacts in a general decrease of DNA methylation, particularly at the *Bdnf* gene (Gassen et al., 2015a). Such regulation of BDNF expression has been implicated in the development and treatment of psychiatric diseases, aging and carcinogenesis (Kang et al., 2015a, 2015b; Kim et al., 2015; McKinney et al., 2015; Mendelsohn and Larrick, 2012). In short, all of these observations lead to postulate that FKBP51 may be a sort of hub factor able to link varied mechanisms involving differentiation, developmental, endocrine, psychiatric and oncologic processes.

The immunosuppressant macrolide FK506 has been proposed for anticancer treatments due to its activity targeting FKBP proteins (Romano et al., 2010c). The importance of TERT in tumor biology has led to many efforts during the last two decades to develop anticancer therapies that target telomerase. Unfortunately, results were disappointing because of the failure of the strategies to specifically target telomerase function (Maida and Masutomi, 2015). In this sense, it is interesting the fact that FK506 significantly inhibits telomerase activity (Figure 4C), raising the possibility that drugs able to target FKBP51 may lead to growth arrest and apoptosis in cancer cells. The recent development of FKBP51 specific ligands able to discriminate this immunophilin among others (Gaali et al., 2015), including the highly homologous FKBP52, provides a strong stimulus to focus future efforts on the elucidation of the molecular mechanism of action of FKBP51 and the therapeutic use of small molecules to sensitize cancer cells or resistant variants of cancer cells to chemotherapy.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A.
Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molonc.2016.05.002.

REFERENCES

Aguirre-Ghiso, J.A., Frankel, P., Farias, E.F., Lu, Z., Jiang, H., Olsen, A., Feig, L.A., de Kier Joffe, E.B., Foster, D.A., 1999. RalA requirement for v-Src- and v-Ras-induced tumorigenicity and overproduction of urokinase-type plasminogen activator: involvement of metalloproteases. Oncogene 18, 4718–4725.

Banerjee, A., Periyasamy, S., Wolf, I.M., Hinds Jr., T.D., Yong, W., Shou, W., Sanchez, E.R., 2008. Control of glucocorticoid and progesterone receptor subcellular localization by the ligand-binding domain is mediated by distinct interactions with tetratricopeptide repeat proteins. Biochemistry 47, 10471–10480.

Baughman, G., Wiederrecht, G.J., Campbell, N.F., Martin, M.M., Bourgeois, S., 1995. FKBP51, a novel T-cell-specific immunophilin capable of calcineurin inhibition. Mol. Cell Biol. 15, 4395–4402.

Beliakoff, J., Whitesell, L., 2004. Hsp90: an emerging target for breast cancer therapy. Anticancer Drugs 15, 651–662.

Borghi, A., Verstrepen, L., Beyaert, R., 2016. TRAF2 multitasking in TNF receptor-induced signaling to NF-kappaB, MAP kinases and cell death. Biochem. Pharmacol.

Colo, G.P., Rubio, M.F., Nojek, I.M., Werbajh, S.E., Echeverria, P.C., Alvarado, C.V., Nahmod, V.E., Galigniana, M.D., Costas, M.A., 2008. The p160 nuclear receptor co-activator RAC3 exerts an anti-apoptotic role through a cytoplasmatic action. Oncogene 27, 2430–2444.

Chambraud, B., Belabes, H., Fontaine-Lenoir, V., Fellous, A., Baulieu, E.E., 2007. The immunophilin FKBP52 specifically binds to tubulin and prevents microtubule formation. FASEB J. 21, 2787–2797.

Chung, J., Khadka, P., Chung, I.K., 2012. Nuclear import of hTERT requires a bipartite nuclear localization signal and Akt-mediated phosphorylation. J. Cell Sci. 125, 2684–2697.

Davies, T.H., Ning, Y.M., Sanchez, E.R., 2002. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J. Biol. Chem. 277, 4597–4600.

DeZwaan, D.C., Freeman, B.C., 2010. HSP90 manages the ends. Trends Biochem. Sci. 35, 384–391.

Echeverria, P.C., Mazaira, G., Erlejman, A., Gomez-Sanchez, C., Piwien Pilipuk, G., Galigniana, M.D., 2009. Nuclear import of the glucocorticoid receptor-hsp90 complex through the nuclear pore complex is mediated by its interaction with Nup62 and importin beta. Mol. Cell Biol. 29, 4788–4797.

Eisenstein, M., 2011. Telomeres: all's well that ends well. Nature 478, S13–S15.

Ellsworth, K.A., Eckloff, B.W., Li, L., Moon, I., Fridley, B.L., Jenkins, G.D., Carlson, E., Brisbin, A., Abo, R., Bamlet, W., Petersen, G., Wieben, E.D., Wang, L., 2013. Contribution of FKBP5 genetic variation to gemcitabine treatment and survival in pancreatic adenocarcinoma. PLoS One 8, e70216.

Erlejman, A.G., De Leo, S.A., Mazaira, G.I., Molinari, A.M., Camisay, M.F., Fontana, V., Cox, M.B., Piwien-Pilipuk, G., Galigniana, M.D., 2014a. NF-kappaB transcriptional activity is modulated by FK506-binding proteins FKBP51 and FKBP52: a role for peptidyl-prolyl isomerase activity. J. Biol. Chem. 289, 26263–26276.

Erlejman, A.G., Lagadari, M., Galigniana, M.D., 2013. Hsp90-binding immunophilins as a potential new platform for drug treatment. Future Med. Chem. 5, 591–607.

Erlejman, A.G., Lagadari, M., Harris, D.C., Cox, M.B., Galigniana, M.D., 2014b. Molecular chaperone activity and biological regulatory actions of the TPR-domain immunophilins FKBP51 and FKBP52. Curr. Protein Pept. Sci. 15, 205–215.

Feng, J., Funk, W.D., Wang, S.S., Weinrich, S.L., Avilion, A.A., Chiu, C.P., Adams, R.R., Chang, E., Allsopp, R.C., Yu, J., et al., 1995. The RNA component of human telomerase. Science 269, 1236–1241.

Forsythe, H.L., Jarvis, J.L., Turner, J.W., Elmore, L.W., Holt, S.E., 2001. Stable association of hsp90 and p23, but not hsp70, with active human telomerase. J. Biol. Chem. 276, 15571–15574.

Gaali, S., Kirschner, A., Cuboni, S., Hartmann, J., Kozany, C., Balsevich, G., Namendorf, C., Fernandez-Vizarra, P., Sippel, C.,

Zannas, A.S., Draenert, R., Binder, E.B., Almeida, O.F., Ruhter, G., Uhr, M., Schmidt, M.V., Touma, C., Bracher, A., Hausch, F., 2015. Selective inhibitors of the FK506-binding protein 51 by induced fit. Nat. Chem. Biol. 11, 33—37.

Galigniana, M.D., 1998. Native rat kidney mineralocorticoid receptor is a phosphoprotein whose transformation to a DNA-binding form is induced by phosphatases. Biochem. J. 333 (Pt 3), 555—563.

Galigniana, M.D., 2012. Steroid receptor coupling becomes nuclear. Chem. Biol. 19, 662—663.

Galigniana, M.D., Echeverria, P.C., Erlejman, A.G., Piwien-Pilipuk, G., 2010a. Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore. Nucleus 1, 299—308.

Galigniana, M.D., Erlejman, A.G., Monte, M., Gomez-Sanchez, C., Piwien-Pilipuk, G., 2010b. The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events. Mol. Cell Biol. 30, 1285—1298.

Gallo, L.I., Ghini, A.A., Piwien Pilipuk, G., Galigniana, M.D., 2007. Differential recruitment of tetratricorpeptide repeat domain immunophilins to the mineralocorticoid receptor influences both heat-shock protein 90-dependent retrotransport and hormone-dependent transcriptional activity. Biochemistry 46, 14044—14057.

Gallo, L.I., Lagadari, M., Piwien-Pilipuk, G., Galigniana, M.D., 2011. The 90-kDa heat-shock protein (Hsp90)-binding immunophilin FKBP51 is a mitochondrial protein that translocates to the nucleus to protect cells against oxidative stress. J. Biol. Chem. 286, 30152—30160.

Gassen, N.C., Fries, G.R., Zannas, A.S., Hartmann, J., Zschocke, J., Hafner, K., Carrillo-Roa, T., Steinbacher, J., Preissinger, S.N., Hoeijmakers, L., Knop, M., Weber, F., Kloiber, S., Lucae, S., Chrousos, G.P., Carell, T., Ising, M., Binder, E.B., Schmidt, M.V., Ruegg, J., Rein, T., 2015a. Chaperoning epigenetics: FKBP51 decreases the activity of DNMT1 and mediates epigenetic effects of the antidepressant paroxetine. Sci. Signal 8, ra119.

Gassen, N.C., Hartmann, J., Schmidt, M.V., Rein, T., 2015b. FKBP5/ FKBP51 enhances autophagy to synergize with antidepressant action. Autophagy 11, 578—580.

Gassen, N.C., Hartmann, J., Zannas, A.S., Kretzschmar, A., Zschocke, J., Maccarrone, G., Hafner, K., Zellner, A., Kollmannsberger, L.K., Wagner, K.V., Mehta, D., Kloiber, S., Turck, C.W., Lucae, S., Chrousos, G.P., Holsboer, F., Binder, E.B., Ising, M., Schmidt, M.V., Rein, T., 2016. FKBP51 inhibits GSK3beta and augments the effects of distinct psychotropic medications. Mol. Psychiatry 21, 277—289.

Gassen, N.C., Hartmann, J., Zschocke, J., Stepan, J., Hafner, K., Zellner, A., Kirmeier, T., Kollmannsberger, L., Wagner, K.V., Dedic, N., Balsevich, G., Deussing, J.M., Kloiber, S., Lucae, S., Holsboer, F., Eder, M., Uhr, M., Ising, M., Schmidt, M.V., Rein, T., 2014. Association of FKBP51 with priming of autophagy pathways and mediation of antidepressant treatment response: evidence in cells, mice, and humans. PLoS Med. 11, e1001755.

Guy, N.C., Garcia, Y.A., Sivils, J.C., Galigniana, M.D., Cox, M.B., 2015. Functions of the Hsp90-binding FKBP immunophilins. Subcell Biochem. 78, 35—68.

Ho, S., Clipstone, N., Timmermann, L., Northrop, J., Graef, I., Fiorentino, D., Nourse, J., Crabtree, G.R., 1996. The mechanism of action of cyclosporin A and FK506. Clin. Immunol. Immunopathol. 80, S40—S45.

Holt, S.E., Aisner, D.L., Baur, J., Tesmer, V.M., Dy, M., Ouellette, M., Trager, J.B., Morin, G.B., Toft, D.O., Shay, J.W., Wright, W.E., White, M.A., 1999. Functional requirement of p23 and Hsp90 in telomerase complexes. Genes Dev. 13, 817—826.

Holliday, R., 1996. Endless quest. Bioessays 18, 3—5.

Hukezalie, K.R., Wong, J.M., 2013. Structure-function relationship and biogenesis regulation of the human telomerase holoenzyme. FEBS J. 280, 3194—3204.

Jinwal, U.K., Koren 3rd, J., Borysov, S.I., Schmid, A.B., Abisambra, J.F., Blair, L.J., Johnson, A.G., Jones, J.R., Shults, C.L., O'Leary 3rd, J.C., Jin, Y., Buchner, J., Cox, M.B., Dickey, C.A., 2010. The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules. J. Neurosci. 30, 591—599.

Kang, H.J., Kim, J.M., Bae, K.Y., Kim, S.W., Shin, I.S., Kim, H.R., Shin, M.G., Yoon, J.S., 2015a. Longitudinal associations between BDNF promoter methylation and late-life depression. Neurobiol. Aging 36 (1764), e1761—1767.

Kang, H.J., Kim, J.M., Kim, S.Y., Kim, S.W., Shin, I.S., Kim, H.R., Park, M.H., Shin, M.G., Yoon, J.H., Yoon, J.S., 2015b. A longitudinal study of BDNF promoter methylation and depression in breast Cancer. Psychiatry Investig. 12, 523—531.

Kim, J.M., Kang, H.J., Kim, S.Y., Kim, S.W., Shin, I.S., Kim, H.R., Park, M.H., Shin, M.G., Yoon, J.H., Yoon, J.S., 2015. BDNF promoter methylation associated with suicidal ideation in patients with breast cancer. Int. J. Psychiatry Med. 49, 75—94.

Kim, Y.S., Kim, Y.J., Lee, J.M., Kim, E.K., Park, Y.J., Choe, S.K., Ko, H.J., Kang, C.Y., 2012. Functional changes in myeloid-derived suppressor cells (MDSCs) during tumor growth: FKBP51 contributes to the regulation of the immunosuppressive function of MDSCs. J. Immunol. 188, 4226—4234.

Komura, E., Chagraoui, H., Mansat de Mas, V., Blanchet, B., de Sepulveda, P., Larbret, F., Larghero, J., Tulliez, M., Debili, N., Vainchenker, W., Giraudier, S., 2003. Spontaneous STAT5 activation induces growth factor independence in idiopathic myelofibrosis: possible relationship with FKBP51 overexpression. Exp. Hematol. 31, 622—630.

Lagadari, M., De Leo, S.A., Camisay, M.F., Galigniana, M.D., Erlejman, A.G., 2015. Regulation of NF-kappaB signalling cascade by immunophilins. Curr. Mol. Pharmacol. 9, 99—108.

Lee, J.H., Khadka, P., Baek, S.H., Chung, I.K., 2010. CHIP promotes human telomerase reverse transcriptase degradation and negatively regulates telomerase activity. J. Biol. Chem. 285, 42033—42045.

Li, T.K., Baksh, S., Cristillo, A.D., Bierer, B.E., 2002. Calcium- and FK506-independent interaction between the immunophilin FKBP51 and calcineurin. J. Cell Biochem. 84, 460—471.

Liu, T.M., Martina, M., Hutmacher, D.W., Hui, J.H., Lee, E.H., Lim, B., 2007. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. Stem Cells 25, 750—760.

Maida, Y., Masutomi, K., 2015. Telomerase reverse transcriptase moonlights: therapeutic targets beyond telomerase. Cancer Sci. 106, 1486—1492.

Manda, G., Isvoranu, G., Comanescu, M.V., Manea, A., Debelec Butuner, B., Korkmaz, K.S., 2015. The redox biology network in cancer pathophysiology and therapeutics. Redox Biol. 5, 347—357.

Manders, E.M., Verbeek, F.J., Aten, J.A., 1993. Measurement of colocalization of objects in dual-color confocal images. J. Microsc. 169, 375—382.

Massard, C., Zermati, Y., Pauleau, A.L., Larochette, N., Metivier, D., Sabatier, L., Kroemer, G., Soria, J.C., 2006. hTERT: a novel endogenous inhibitor of the mitochondrial cell death pathway. Oncogene 25, 4505—4514.

Mazaira, G.I., Camisay, M.F., De Leo, S., Erlejman, A.G., Galigniana, M.D., 2016. Biological relevance of Hsp90-binding immunophilins in cancer development and treatment. Int. J. Cancer 138, 797—808.

Mazaira, G.I., Lagadari, M., Erlejman, A.G., Galigniana, M.D., 2014. The emerging role of TPR-domain immunophilins in the

mechanism of action of steroid receptors. Nucl. Receptor Res 1, 17, 101094. http://dx.doi.org/10.11131/2014/101094.

McKinney, B.C., Lin, C.W., Oh, H., Tseng, G.C., Lewis, D.A., Sibille, E., 2015. Hypermethylation of BDNF and SST genes in the orbital frontal cortex of older individuals: a putative mechanism for declining gene expression with age. Neuropsychopharmacology 40, 2604–2613.

Mendelsohn, A.R., Larrick, J.W., 2012. Epigenetic-mediated decline in synaptic plasticity during aging. Rejuvenation Res. 15, 98–101.

Menicanin, D., Bartold, P.M., Zannettino, A.C., Gronthos, S., 2009. Genomic profiling of mesenchymal stem cells. Stem Cell Rev 5, 36–50.

Meyerson, M., Counter, C.M., Eaton, E.N., Ellisen, L.W., Steiner, P., Caddle, S.D., Ziaugra, L., Beijersbergen, R.L., Davidoff, M.J., Liu, Q., Bacchetti, S., Haber, D.A., Weinberg, R.A., 1997. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 90, 785–795.

Mukaide, H., Adachi, Y., Taketani, S., Iwasaki, M., Koike-Kiriyama, N., Shigematsu, A., Shi, M., Yanai, S., Yoshioka, K., Kamiyama, Y., Ikehara, S., 2008. FKBP51 expressed by both normal epithelial cells and adenocarcinoma of colon suppresses proliferation of colorectal adenocarcinoma. Cancer Invest. 26, 385–390.

Nair, S.C., Rimerman, R.A., Toran, E.J., Chen, S., Prapapanich, V., Butts, R.N., Smith, D.F., 1997. Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. Mol. Cell Biol. 17, 594–603.

Ni, L., Yang, C.S., Gioeli, D., Frierson, H., Toft, D.O., Paschal, B.M., 2010. FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. Mol. Cell Biol. 30, 1243–1253.

Pei, H., Li, L., Fridley, B.L., Jenkins, G.D., Kalari, K.R., Lingle, W., Petersen, G., Lou, Z., Wang, L., 2009. FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. Cancer Cell 16, 259–266.

Periyasamy, S., Hinds Jr., T., Shemshedini, L., Shou, W., Sanchez, E.R., 2010. FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A. Oncogene 29, 1691–1701.

Pratt, W.B., Galigniana, M.D., Morishima, Y., Murphy, P.J., 2004. Role of molecular chaperones in steroid receptor action. Essays Biochem. 40, 41–58.

Quinta, H.R., Galigniana, M.D., 2012. The neuroregenerative mechanism mediated by the Hsp90-binding immunophilin FKBP52 resembles the early steps of neuronal differentiation. Br. J. Pharmacol. 166, 637–649.

Quintá, H.R., Maschi, D., Gomez-Sanchez, C., Piwien-Pilipuk, G., Galigniana, M.D., 2010. Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth. J. Neurochem. 115, 716–734.

Ratajczak, T., Cluning, C., Ward, B.K., 2015. Steroid receptor-associated immunophilins: a gateway to steroid signalling. Clin. Biochem. Rev. 36, 31–52.

Ratajczak, T., Ward, B.K., Minchin, R.F., 2003. Immunophilin chaperones in steroid receptor signalling. Curr. Top Med. Chem. 3, 1348–1357.

Romano, S., D'Angelillo, A., Pacelli, R., Staibano, S., De Luna, E., Bisogni, R., Eskelinen, E.L., Mascolo, M., Cali, G., Arra, C., Romano, M.F., 2010a. Role of FK506-binding protein 51 in the control of apoptosis of irradiated melanoma cells. Cell Death Differ. 17, 145–157.

Romano, S., D'Angelillo, A., Staibano, S., Ilardi, G., Romano, M.F., 2010b. FK506-binding protein 51 is a possible novel tumoral marker. Cell Death Dis. 1, e55.

Romano, S., Di Pace, A., Sorrentino, A., Bisogni, R., Sivero, L., Romano, M.F., 2010c. FK506 binding proteins as targets in anticancer therapy. Anticancer Agents Med. Chem. 10, 651–656.

Romano, S., Mallardo, M., Romano, M.F., 2011a. FKBP51 and the NF-kappaB regulatory pathway in cancer. Curr. Opin. Pharmacol. 11, 288–293.

Romano, S., Sorrentino, A., Di Pace, A.L., Nappo, G., Mercogliano, C., Romano, M.F., 2011b. The emerging role of large immunophilin FK506 binding protein 51 in cancer. Curr. Med. Chem. 18, 5424–5429.

Romano, S., Staibano, S., Greco, A., Brunetti, A., Nappo, G., Ilardi, G., Martinelli, R., Sorrentino, A., Di Pace, A., Mascolo, M., Bisogni, R., Scalvenzi, M., Alfano, B., Romano, M.F., 2013. FK506 binding protein 51 positively regulates melanoma stemness and metastatic potential. Cell Death Dis. 4, e578.

Romano, S., Xiao, Y., Nakaya, M., D'Angelillo, A., Chang, M., Jin, J., Hausch, F., Masullo, M., Feng, X., Romano, M.F., Sun, S.C., 2015. FKBP51 employs both scaffold and isomerase functions to promote NF-kappaB activation in melanoma. Nucleic Acids Res. 43, 6983–6993.

Salatino, M., Beguelin, W., Peters, M.G., Carnevale, R., Proietti, C.J., Galigniana, M.D., Vedoy, C.G., Schillaci, R., Charreau, E.H., Sogayar, M.C., Elizalde, P.V., 2006. Progestin-induced caveolin-1 expression mediates breast cancer cell proliferation. Oncogene 25, 7723–7739.

Seimiya, H., Sawada, H., Muramatsu, Y., Shimizu, M., Ohko, K., Yamane, K., Tsuruo, T., 2000. Involvement of 14-3-3 proteins in nuclear localization of telomerase. EMBO J. 19, 2652–2661.

Sivils, J.C., Storer, C.L., Galigniana, M.D., Cox, M.B., 2011. Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52). Curr. Opin. Pharmacol. 11, 314–319.

Stechschulte, L.A., Hinds Jr., T.D., Ghanem, S.S., Shou, W., Najjar, S.M., Sanchez, E.R., 2014. FKBP51 reciprocally regulates GRalpha and PPARgamma activation via the Akt-p38 pathway. Mol. Endocrinol. 28, 1254–1264.

Stechschulte, L.A., Sanchez, E.R., 2011. FKBP51-a selective modulator of glucocorticoid and androgen sensitivity. Curr. Opin. Pharmacol. 11, 332–337.

Storer, C.L., Dickey, C.A., Galigniana, M.D., Rein, T., Cox, M.B., 2011. FKBP51 and FKBP52 in signaling and disease. Trends Endocrinol. Metab. 22, 481–490.

Thorne, J.L., Campbell, M.J., 2015. Nuclear receptors and the Warburg effect in cancer. Int. J. Cancer 137, 1519–1527.

Toneatto, J., Guber, S., Charo, N.L., Susperreguy, S., Schwartz, J., Galigniana, M.D., Piwien-Pilipuk, G., 2013. Dynamic mitochondrial-nuclear redistribution of the immunophilin FKBP51 is regulated by the PKA signaling pathway to control gene expression during adipocyte differentiation. J. Cell Sci. 126, 5357–5368.

Weiwad, M., Edlich, F., Kilka, S., Erdmann, F., Jarczowski, F., Dorn, M., Moutty, M.C., Fischer, G., 2006. Comparative analysis of calcineurin inhibition by complexes of immunosuppressive drugs with human FK506 binding proteins. Biochemistry 45, 15776–15784.

Wochnik, G.M., Ruegg, J., Abel, G.A., Schmidt, U., Holsboer, F., Rein, T., 2005. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J. Biol. Chem. 280, 4609–4616.

Xu, X., Su, B., Barndt, R.J., Chen, H., Xin, H., Yan, G., Chen, L., Cheng, D., Heitman, J., Zhuang, Y., Fleischer, S., Shou, W., 2002. FKBP12 is the only FK506 binding protein mediating T-cell inhibition by the immunosuppressant FK506. Transplantation 73, 1835–1838.