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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

Abbreviations: FKBP51, FK506-binding protein of 51-kDa; FKBP52, FK506-binding protein of 52-kDa; CyP, cyclophilin; Hsp90, heat-shock protein of 90-kDa; Hsp70, heat-shock protein of 70-kDa; hTERT, human reverse transcriptase subunit of telomerase; TPR, tetratricopeptide repeats; PPIase, peptidyl-prolyl-(*cis/trans*)-isomerase; Rad, radicicol; H2DCF-DA, 2′,7′-dichloro-dihydrofluorescein diacetate; BDNF, brain-derived neurotrophic factor; DNMT1, DNA-methyl transferase 1.

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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 prooncogenic 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.