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Biological relevance of Hsp90-binding immunophilins in cancer development and treatment

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Mini Review

Immunophilins are a family of intracellular receptors for immunosuppressive drugs. Those immunophilins that are related to immunosuppression are the smallest proteins of the family, *i.e.*, FKBP12 and CyPA, whereas the other members of the family have higher molecular weight because the show additional domains to the drug-binding site. Among these extra domains, the TPR-domain is perhaps the most relevant because it permits the interaction of high molecular weight immunophilins with the 90-kDa heat-shock protein, Hsp90. This essential molecular chaperone regulates the biological function of several protein-kinases, oncogenes, protein phosphatases, transcription factors and cofactors . Hsp90-binding immunophilins where first characterized due to their association with steroid receptors. They regulate the cytoplasmic transport and the subcellular localization of these and other Hsp90 client proteins, as well as transcriptional activity, cell proliferation, cell differentiation and apoptosis. Hsp90-binding immunophilins are frequently overexpressed in several types of cancers and play a key role in cell survival. In this article we analyze the most important biological actions of the best characterized Hsp90-binding immunophilins in both steroid receptor function and cancer development and discuss the potential use of these immunophilins for therapeutic purposes as potential targets of specific small molecules.

Immunophilins form a family of molecular chaperones that show rotamase or peptidyl-prolyl-(*cis/trans*)-isomerase (PPIase) activity, *i.e.,* the reversible *cis↔trans* interconvertion of Xaa-Pro bonds.[1] These are abundant foldases in all cell types and are classified into two subfamilies according to their ability to bind specific immunosuppressant drugs[2]—Cyclophilins when they bind the cyclic undecapeptide cyclosporine A (CsA) or *FK506-Binding Proteins* (FKBPs) when they bind the macrolide FK506 (or tacrolimus). Table 1 shows a list of the most relevant abbreviations used in this work and a brief description of the biological actions of each factor.

The signature domain of immunophilins is the PPIase domain, which is also the drug binding domain. Only the smallest members of each subfamily, CyPA and FKBP12, are related to the immunosuppressive action. This takes place when the respective drug•immunophilin complex inhibits the Ser/Thr-phosphatase activity of PP2B/calcineurin preventing the dephosphorylation of the transcription factor Nuclear Factor of Activated T cells and its subsequent nuclear translocation. Therefore, the production of interleukines and interferon-γ is inhibited.[3] Some members of the FKBP subfamily also bind other macrolide, rapamycin (or sirolimus). However, FKBP12•rapamycin complexes show a different mechanism of action; they target mTOR (Mammalian Target of Rapamycin), a Ser/Thr protein kinase able to regulate cell proliferation, cell growth, cell motility and protein synthesis.[4]

High molecular weight immunophilins have a more complex architecture than that shown by the two immunosuppressive members of the family (Fig. 1) and are not related to the immunosupression process. In addition to the PPIase domain, they also have the nucleotide-binding domain, where ATP binds, the calmodulin-binding domain, a poorly characterized domain able to interact with calmodulin and sequences of 34 amino acids repeated *in tandem*, the TPR domains,

Key words: FKBP51, FKBP52, FKBP38, FKBPL, NF-κB, steroid receptor

Abbreviations: AR: androgen receptor; CsA: cyclosporine A; CyP: cyclophilins; ER: estrogen receptor; FKBP: FK506-binding protein; FKBPL/WisP39: FK506-binding protein-like/WAF1-CIP1-stabilizing protein 39; GR: glucocorticoid receptor; Hsp90: heat-shock protein of 90-kDa; IκB: inhibitor of *kB*; IKK: IκB kinases; mTOR: mammalian target of rapamycin; MR: mineralocorticoid receptor; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PP5: protein-phosphatase 5; PPIase: peptidylprolyl isomerase; PR: progesterone receptor; TDO: tryptophan-2,3-dioxygenase; TPR: tetratricopeptide repeats

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Table 1. Nomenclature and biological role of some factors related to immunophilins

Abbreviation	Full or conventional name	Property
FK506	Tacrolimus, Fujimicyn	Macrolide lactone used as immunosuppressive drug. FKBP12•FK506 complexes inhibit the enzymatic activity of PP2B/calcineurin.
PPIase	Peptidyprolyl isomerase	Enzymatic activity of rotamase (cis/trans isomerization of X-Propeptide bonds).
mTOR	mTOR	Ser/Thr kinase signalling cascade regulated by FKBP12•Rapamycin complexes.
FKBP	FK506-Binding Protein	Subfamily of immunophilins showing a PPIase domain where FK506 binds.
TPR	Tetratricopeptide repeats	Degenerate 34 amino acid sequence motif involved in protein-protein interactions.
Hsp90	Heat-shock protein of 90-kDa	Forms complexes with TPR-domain immunophilins (one TPR protein per Hsp90 dimer).
Hop/p60	Heat-shock organizing protein	TPR domain protein that favors the formation of complexes between Hsp90 and Hsp70.
RAC3	Receptor-associated coactivator 3	Coactivator of steroid receptors that is recruited to nuclear complexes in several tumors.
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells	Family of proteins with structural similarity to the retroviral oncoprotein v-Rel that shows transcriptional activity properties.
IκB	Inhibitor of *kB*	Family of inhibitory proteins showing different affinities for individual NF-κB complexes.
IKK	IκB kinase	Family of IκB kinases that favors IκB dissociation from NF-κB and its nuclear relocalization.
TDO	TDO	Degradation of tryptophan to kynurenine, which favors the activation of the aryl hydrocarbon recep tor and consequently tumor cell proliferation and invasiveness.

through which they interact with the 90-kDa heat-shock protein, Hsp90. The Hsp90-binding immunophilins were first described as members of the steroid receptor heterocomplex,[5] these immunophilins being the best characterized of this group—FKBP52 (gene name *fkbp4*), FKBP51 (gene name *fkbp5*), CyP40 (gene name *ppID*) and the FKBP-like proteins (which show no enzymatic activity) PP5 (gene name *ppp5C*) and FKBPL/WisP39 (gene name *fkbpl*). All of these immunophilins have their counter-part in plants,[6] suggesting a conserved function during the evolution.

Hsp90-Binding Immunophilins and Transcription Factors

All steroid receptors exist as oligomeric heterocomplexes.[5] Early evidence already showed that the presence of the Hsp90 dimer plays a cardinal role in these complexes.[7–10] The first evidence that the immunophilin FKBP52 (first called p59 and Hsp56) was bound to Hsp90 in nontransformed receptors can be traced back to the early '90 s.[11] This immunophilin is present in mature rather than intermediate receptor complexes. In nontransformed isoforms, the stoichiometry of the receptor•$(Hsp90)_2$ complex shows one molecule of Hsp70, one molecule of p23 and a TPR-domain cochaperone bound to the TPR acceptor site of the Hsp90 dimer.[12,13] During the early steps of heterocomplex assembly, the TPR protein Hop/p60/Sti1 is required for bringing together Hsp90 and Hsp70, but after the formation of the complex and its subsequent transference to the aporeceptor, Hop/p60/Sti1 is released and a TPR-domain immunophilin occupies the TPR acceptor site on the Hsp90 dimer. FKBP51 is the immunophilin present in the mature and transcriptionally inactive receptor and is exchanged by FKBP52 upon steroid binding (Fig. 2). Because the PPIase domain of FKBP52, but not the PPIase domain of FKBP51, is able to interact with dynein/dynactin,[14,15] the motor protein complex is also recruited to steroid receptors and powers the active cytoplasmic transport to the nucleus along cytoskeletal tracks.[12,13]

Hsp90 is mostly localized in the cytoplasm of unstressed cells and a small fraction is nuclear. In early times there was a controversy related to the fact that Hsp90 could not exist associated to the nuclear pool of steroid receptors.[16,17] Nonetheless, this notion contradicted experiments showing cross-linking of Hsp90 with PR in nuclear extracts[18] and more importantly, crosslinking with ER in intact cells,[19] demonstrating that both receptors form nuclear heterocomplexes with Hsp90 in the absence of agonist. Today, it is accepted that this is the case and that Hsp90 complexes are able to be assembled with steroid receptors in the nuclear compartment.[20,21]

The classic model for steroid receptor activation usually described in the literature was posed in the literature even

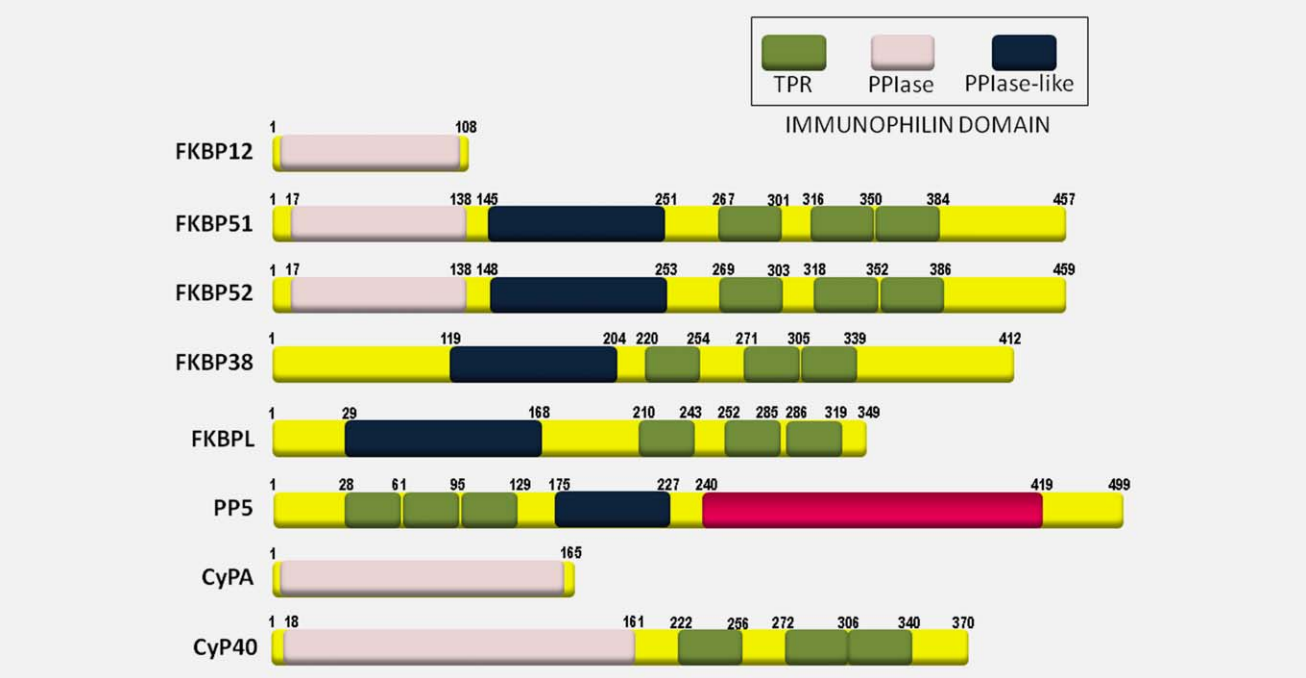

Figure 1. Structural domains of some Hsp90-binding immunophilins belonging to the FKBP and cyclophilin (CyP) subfamilies compared with their respective archetype members responsible for the immunosuppression action, FKBP12 and CyPA. Note that these two immunophilins only show the PPIase domain, whereas those that are capable to interact with Hsp90 have multiple repetitions of the TPR domain. PPIase-like domains preserve structural homology with the PPIase domain, but they lack enzymatic activity of peptidylprolyl isomerase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

before the time of those findings[22,23] and postulated the heuristic notion that Hsp90 anchors the receptor to cytoplasmic structures. The release of the chaperone (a process usually referred to as "transformation") was thought to be the essential requirement for receptor translocation to the nucleus. While Gasc *et al.*[24] showed that Hsp90 and FKBP52 are bound to PR in the nucleus, recent experimental evidence demonstrates that the Hsp90•FKBP52 complex is not dissociated from steroid receptors upon steroid binding, but it is also required for the cytoplasmic retrotransport of the receptor.[14] Moreover, the chaperone complex also facilitates the passage of the whole steroid receptor heterocomplex through the nuclear pore interacting with structures of the pore such as nucleoporins.[25] Therefore, the transformation processes leading to receptor dimerization is predicted to be a nuclear event rather than an early cytoplasmic step. Very recent publications have confirmed these findings and have demonstrated that steroid receptor dimerization occurs in the nucleus and not in the cytoplasm,[26,27] as the novel model predicts.

Other Hsp90-binding immunophilins commonly associated to steroid receptors are CyP40, PP5 and FKBPL/WisP39. CyP40 is a member of the cyclophilin subfamily and is usually found associated to ER and PR rather than to GR and MR,[28–30] whereas PP5 is an immunophilin-like protein that shows Ser/Thr-protein-phosphatase activity with pro-proliferative actions in most cells.[31] Both Hsp90-binding immunophilins are also able to interact with dyenin motors suggesting a possible redundancy with FKBP52 as protein carrying factors. FKBPL/WisP39 is other immunophilin-like protein that was originally found during screening for genes that were protective against ionizing radiation.[32,33] It is most closely related to FKBP52 and also shows the ability to interact with Hsp90 in steroid receptor complexes, sharing with FKBP52 exactly the same properties for the cytoplasmic retrotransport of the GR.[34,35] Table 2 summarizes the most relevant features of the immunophilins analyzed in this work.

Most of the members of the Hsp90-binding immunophilin family also form complexes with other transcription factors and protein kinases related to the regulation of the cell cycle.[36] Among them, p53 cytoplasmic mutants are associated to Hsp90 and FKBP52, this chaperone complex also being responsible for the retrotransport of the proapoptotic factor *via* dynein/dynactin motor proteins.[37] The p160 nuclear receptor coactivator family member RAC3 (*Receptor-Associated Coactivator-3*), which is recruited by steroid receptors[38] and other factors whose expression is related to several tumors (*i.e.,* NF-κB,[39] E2F,[40] AP-1,[41] STAT6,[42] etc.) is also able to interact with the Hsp90•FKBP52•dynein heterocomplex, a molecular transport machinery that is also responsible for RAC3 cytoplasmic retrotransport.[43]

Recently, it was demonstrated that NF-κB is regulated by FKBP51 and FKBP52 in an antagonistic manner.[44] FKBP51 is an inhibitory factor of NF-κB overall action, whose activity is not dependent on its PPIase activity. However, FKBP52 is a strong activator, a role where the PPIase enzymatic activity

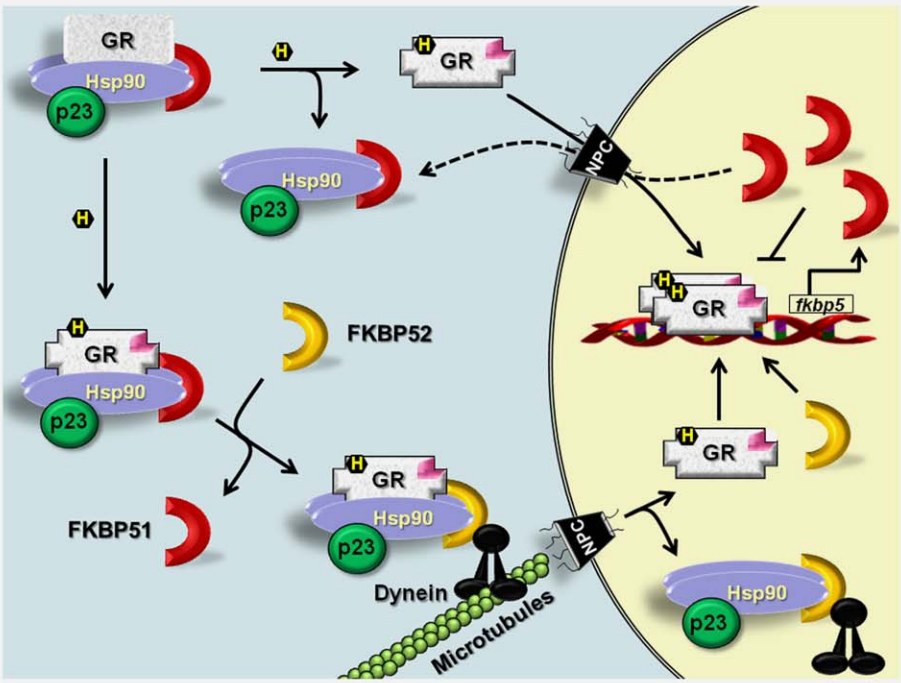

Figure 2. Molecular mechanism of action of the glucocorticoid receptor. According to the conjectural classic model, the Hsp90-based heterocomplex is dissociated from GR upon hormone (H) binding (upper part of the model). This permits a conformational change in the receptor exposing its nuclear localization signal (colored pink corner). After receptor diffusion throughout the cytoplasm, it is translocated to the nucleus and transcription takes place. The experimentally proved modern model sustains that steroid binding promotes the exchange of the Hsp90-binding immunophilin FKBP51 by FKBP52, which is able to interact with dynein motors and also shows a positive regulation of the transcriptional activity. The whole GR•Hsp90•FKBP52 complex is actively transported towards the nucleus using microtubules tracks and translocates through the nuclear pore complex (NPC), such that transformation (Hsp90 release) occurs in the nucleoplasm. Note that the novel model predicts that GR dimerization must be a nuclear event, as it has recently been proved.[26,153] One of the targets genes for GR is the *fkbp5* gene, which encodes for FKBP51 and generates and ultra-short inhibitory feedback on the receptor action. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

is essential. Interestingly, Hsp90 is not required for these effects and the regulation by immunophilins appears to be direct because purified RelA binds to purified FKBP52.[44] This immunophilin-dependent regulatory mechanism could explain the pleiotropic actions of NF-κB according to the FKBP52 to FKBP51 expression ratio in different cell types and biological circumstances. In view of these and the above-commented properties, immunophilins have become an attractive novel pharmacologic target.[45–47]

FKBP51

The relevance of FKBP51 in steroid receptor signalling was first elucidated in New World Monkeys. In squirrel monkeys, the levels of free cortisol in plasma are two orders of magnitude higher than in other primates, including humans. However these animals do not develop signs of hypercortisolism. This is due to GR resistance in the target organs, which is conferred by overexpression of FKBP51.[48,49] An important feature of the *fkbp5* gene that encodes for FKBP51 is that its expression is induced by glucocorticoids, generating an intracellular ultra-short negative feedback loop for GR activity[50] (Fig. 2). Thus, steroid-activated GR induces *fkbp5* transcription by activation of two intronic hormone responsive elements[51] increasing the expression of FKBP51. At the end, the GR function is greatly impaired by reduction of both GR steroid binding capacity and inhibition of transcriptional activity. Importantly, an impaired signalling cascade *via* cortisol-activated GR leads to an impaired negative feedback regulation, and thus, to partial glucocorticoid resistance. Interestingly, this circuit appears to be one of the most robust biological abnormalities observed in mood disorders[52] and the existence of specific polymorphic isoforms of FKBP51 strongly correlates with the dysregulation of the stress response and the development of post-traumatic stress-disorders.[53,54]

Recently, it was demonstrated that FKBP51 is a mitochondrial protein.[55] About 50% of the cellular pool of this immunophilin localizes in mitochondria in a TPR-domain-dependent manner. FKBP51 undergoes a rapid and reversible nuclear accumulation accompanied by nucleolar concentration under several situation of stress (peroxides, heat-shock, UV light, serum deprivation, high osmolarity of the medium, metals, proinflammatory stimuli, etc.).[55] Overexpression of FKBP51 shows antiapoptotic effects, whereas its knock-down sensitizes cells to programmed cell death. Accordingly, FKBP51 expression is high in most cancer cell lines and in cancer tissues.

Table 2. Relevant immunophilins belonging to the FKBP subfamily in humans

Protein	Aliases	Gene	Cytogenetic band	Ligands	Known biological functions
FKBP12	FKBP1	*fkbp1A*	20p13	FK506	Calcineurin inhibitor[143]. Immunosuppressor factor.[133] TGFβ,[144] IP3[145] and ryanodine receptors[146] modulator.
	PKC12			Rapamycin	
	PPI-1A				
FKBP51	FKBP54	*fkbp5*	6p21.31	FK506	GR,[48] MR,[147] PR[148] transcriptional inhibition and AR activation.[58] IKK[136] and p65/RelA[100] interactor. Impairs dynein-powered dependent retrotransport of nuclear factors.[149] Impairment of neuronal differentiation.[150]
	p54			Rapamycin	
	AIG6			SAFit 1 & 2	
	ARP6				
FKBP52	HBI	*fkbp4*	12p13.33	FK506	Favors ligand binding to steroid receptors[151]and dynein-powered retrotransport.[14] NF-κB activator.[100] Neurotrophic factor.[150] TDO activity suppressor.[94] Copper transport.[152]
	p59			Rapamycin	
	Hsp56				
	FKBP59				
FKBP38	PPIase FKBP8	*fkbp8*	19p12	FK506, GPI1046	Inherent calcineurin inhibitor.[109] Bcl2 regulator.[117] PRL3 inhibitor. Hepatitis C virus interactor.[115] Antiapoptotic factor[109]
	FKBPr38			N-(N′,N′-dimethyl carboxamidomethyl) cycloheximide.	
FKBPL	NG7	*fkbpL*	6p21.3	ALM201	steroid receptor action.[139] Antiangiogenic factor.[127]
	DIR1				
	WISP39				

FKBP51 is regarded as a negative regulator of steroid receptor activity in most studies reported to date[56] except for the case of AR,[57] where the overexpression of FKBP51 increases AR transcriptional activity in the presence or absence of androgens in the medium and siRNA knockdown of the immunophilin strongly impairs AR-dependent gene transcription and cell proliferation.[58,59] The proliferation of prostate cancer cells can be constrained by androgen deprivation therapy accompanied by a therapy with antiandrogens (*e.g.,* bicalutamide) to inhibit AR action by steroid competition with the androgen binding site and displacement of the H12 helix of AR to prevent formation of a productive AF-2 (*Activation Function-2)* binding pocket,[60] a domain that is harbored by the hormone-binding domain and acts as a docking site for coactivators.[61] Therefore, most of the studies have been focused on the endocrine perspective of the gland by testing androgen synthesis inhibitors or AR antagonists (see Ref. [62 for a recent review). Nonetheless, for reasons that are still obscure, this original situation reverts along the time and a castration-resistant prostate cancer is finally developed,[63] raising logical concerns about the efficacy of such therapeutic strategy. In spite of the apparent androgen independence of the tumor, the AR still remains as a critical oncogenic factor that affects both tumor growth and cell survival in the majority of the castration-resistant prostate cancers and about half of the patients with metastatic disease show even a significant amplification of the AR gene, resulting therefore in overexpression of the AR protein.[64,65] Interestingly, an endogenous anti-inflammatory prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 targets the AR and acts as a potent AR inhibitor,[66] rapidly repressing AR target genes, among them, *fkbp5*. This could prevent the positive action of its product, FKBP51, in AR biological actions.

A relevant role for FKBP51 in sustaining cancer cell growth and aggressiveness has been shown in various types of cancers.[66–73] One of the first evidences connecting FKBP51 with malignant pathologies was the observation that this Hsp90-binding immunophilin is overexpressed in idiopathic myelofibrosis,[69] a known chronic myeloproliferative disorder characterized by bone marrow fibrosis and megakaryocyte

hyperplasia. The overexpression of FKBP51 affects the regulation of the growth factor independence of megakaryocyte progenitors and induces resistance to apoptosis. Even though hyperexpression of FKBP51 is observed in several human cancers, including lymphomas, gliomas, melanoma, prostate cancer, etc.,[74] it is down-regulated in other types such as pancreatic cancer.[75] Interestingly, FKBP51 binding to Hsp90 favors the recruitment of the co-chaperone p23 and positively regulates AR signaling[76] and is associated with chemoresistance and radioresistance.[75,77] It has also been shown by siRNA interference studies that FKBP51 suppresses the proliferation of colorectal adenocarcinoma.[78]

All these observations raise the possibility that FKBP51 could be use as a cancer biomarker. In addition, it would be important to investigate common single-nucleotide polymorphisms in the *fkbp5* gene to explore the possibility that, like in the previously referenced case of post-traumatic syndrome disorders, FKBP51 might contribute to individual variations in the biological response to different therapeutic approaches, in particular drug sensitivity. Moreover, the role of FKBP51 in tumourigenesis must be clarified. To date, there are neither clear explanations to justify the fact that FKBP51 functions as an oncogenic factor or a tumor suppressor depending on the tissue type, nor the reasons by which it is down-regulated in pancreatic tumor tissue and is overexpressed in melanomas or lymphomas.

FKBP52

The amino acid sequence of FKBP52 shares 60% identity and 75% similarity with FKBP51.[79,80] The FKBP12-like domains described by Callebaut *et al.*[79] show a good correlation with those present in FKBP51 and FKBP52. These two immunophilins not only share high homology, but they are also quite similar in the organization of their domains and three-dimensional structures.[56] The main functional difference between them lays in the PPIase domain, which is certainly conserved, but residues of the proline-rich loop suspended above the PPIase pocket differ between both proteins affecting protein interactions with larger peptide substrates.[81,82] These differences are responsible for the divergent functions of both proteins. Hence, an FKBP51 mutant containing two point mutations (A116 V and L119P) in the FKBP51 proline-rich loop showed full FKBP52-like activity towards AR.[83] Moreover, the exchange of both PPIase domains in chimeric proteins transforms FKBP52 into FKBP51 and *vice versa*.[15] Thus, the replacement of the N-terminal domain in FKBP52 by that of FKBP51 led to a GR inhibitory immunophilin with highly reduced capacity to bind dynein. Conversely, replacing the PPIase domain of FKBP51 by that of FKBP52 almost completely abolished the inhibitory effect of FKBP51 on GR transcriptional activity and also reconstituted the capacity to interact with dynein.[15]

FKBP52 is overexpressed in breast cancer[84,85] and is also required for normal sexual differentiation and development.[86–88] Accordingly, FKBP52-deficient male mice display characteristics of partial androgen insensitivity syndrome, including dysgenic prostate.[59,89] Interestingly, the BF-3 (*Binding Function-3*) surface of the AR (a region coupled to the AF-2 pocket of the receptor) appears to be the most effective binding site for drugs rather than the AF-2 domain.[90] Accordingly, BF-3 mutations have been identified in patients with prostate cancer or androgen insensitivity syndrome[90–92] and AR X-ray structures showed that BF-3 and AF-2 are structurally attached.[90] Mutations within the BF3 surface of the AR also result in increased dependence on FKBP52 for function. In particular, the region containing Pro723 and Phe673 has been labeled as a putative FKBP52 interaction and/or regulatory surface. It has been shown that the cyclohexane-carboxamide derivative MJC13 specifically inhibits its FKBP52-regulated AR activity by interaction with the BF3 surface,[93] but not due to binding to FKBP52. Nonetheless, FKBP52 function is abrogated, including the ability to translocate AR to the nucleus. Importantly, the secretion of prostate-specific antigen to the medium, the expression of FKBP51 (a positive regulator of AR function) and steroid-dependent proliferation of prostate cancer cells are inhibited, which may have therapeutic implications.

FKBP52 has recently been related to development of glioblastoma multiforme, the most frequent and aggressive primary tumor of the central nervous system.[94] In these tumors, the degradation of tryptophan to kynurenine by tryptophan-2,3-dioxygenase (TDO) favors the constitutive activation of the aryl-hydrocarbon receptor,[95] leading to the inhibition of the antitumor immune response and favoring tumor cell proliferation and invasiveness.[96] FKBP52 knock-down increased TDO constitutive expression and kynurenine production, an effect that seems to be dependent on the PPIase activity of the immunophilin because it is also observed in cells treated with FK506 . This observation raised the possibility that the immunesupressive action of FK506 *via* calcineurin could also be potentiated by increasing the activity of TDO. In a recent study, it was demonstrated that this effect may be GR-dependent.[94]

Other key factor that plays a key role in cancer and inflammation is NF-κB. In unstimulated cells, inactive NF-κB is retained in the cytoplasm due to its association with IκB (Inhibitor of kB), whereas in stimulated cells (stress, TNF, IL-1β. etc.), IκB is dissociated upon phosphorylation by IKKs (IκB kinases) and degraded by the proteasome. This favors the retrograde transport and subsequent nuclear translocation of the active RelA•p50. This heterodimer is the most frequent dimer of NF-κB in most cell types of all tissues[97] and regulates transcription of genes that affect cell proliferation, survival, metastasis and angiogenesis.[98] It has recently been shown that NF-κB retrotransport is significantly impaired by FKBP51 overexpression, whereas FKBP52 favors NF-κB nuclear retention time, a variable directly related to its tumorigenic action.[99] Moreover, the transcriptional response is also regulated by the expression balance of FKBP51 and FKBP52, the former immunophilin being an inhibitory factor

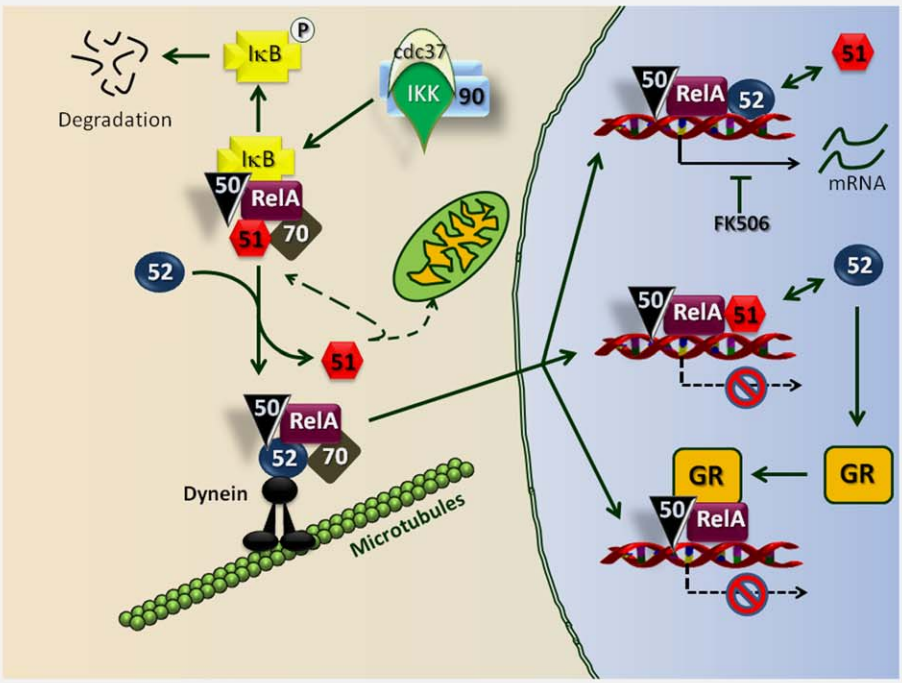

Figure 3. Novel model for the regulation of the biological action of NF-κB by FKBP51 and FKBP52. The p50•RelA complex, the most frequent dimer in all cell types, is associated to FKBP51 and Hsp70 in the cytoplasm. When cells are stimulated, the protein-kinase IKK is activated by phosphorylation via the cdc37•$(Hsp90)_2$ interacting complex, which results in IκB phosphorylation, its dissociation from the NF-κB complex and subsequent degradation via the proteasome. The active NF-κB dimer exchanges immunophilins, such that FKBP51 replaces FKBP52 in the heterocomplex. The latter immunophilin recruits dynein/dynactin motors proteins and mediates the retrotransport of active NF-κB dimers in a PPIase activity-independent manner. FKBP52 is also associated to NF-κB promoter sites of target genes favoring transcriptional activity, an effect that is strongly dependent on the PPIase enzymatic activity of FKBP52 and is consequently prevented by the macrolide FK506. An excess of FKBP51 competes with FKBP52 (↔) and prevents NF-κB biological effects. The steroid-dependent activation of the GR is also favored by FKBP52 because both its cytoplasmic retrotransport and the transcriptional activity mechanisms are favored. Active GR inhibits NF-κB action due to tans-repression mechanisms. Note that FKBP51 is also located in mitochondria, where antiapoptotic effects are favored by this immunophilin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of NF-κB and the latter a strong activator dependent on its PPIase activity[44] (Fig. 3). Actually, ChIP assays have demonstrated that both immunophilins are recruited to the promoter sites of NF-κB target genes and are functionally exchanged upon cell stimulation, such that FKBP52 (the stimulant immunophilin) replaces FKBP51, the negative regulator.[44] Many cancer types exhibit persistent activation of NF-κB, which induces an inflammatory response that is thought to favor cancer development.[99] Therefore, blocking the NF-κB pathway shows therapeutic benefits and one novel and still unexplored strategy to achieve this could be the direct inhibition of immunophilin function.

Figure 3 integrates our recent findings for the role of FKBP51 and FKBP52 on NF-κB signaling.[100] The p50•RelA dimer is associated to FKBP51 in its inactive cytoplasmic state. The Hsp70, an Hsp90 partner chaperone that regulates client proteins functions is also part of this heterocomplex. Upon cell stimulation, the kinase activity of IKK is activated by phosphorylation *via* the cdc37•$(Hsp90)_2$ interacting complex.[101] This results in IκB phosphorylation, dissociation from the NF-κB complex and the subsequent degradation of IκB *via* proteasome. Active NF-κB replaces FKBP51 by FKBP52, an immunophilin that is able to interact with dynein/dynactin motors proteins[102] favoring both the retrotransport of NF-κB[103,104] and its interaction with the nuclear sites of action. FKBP52 greatly favors NF-κB biological action in a PPIase-dependent manner (for example, it is inhibited by FK506 or rapamycin) when the immunophilin is recruited to the promoter sites of NF-κB target genes. On the other hand, the recruitment of FKBP51 to those promoters inhibits the NF-κB biological response. Both immunophilins compete one another and can hamper the original effect of the other. The steroid-dependent activation of the GR, which is also improved by FKBP52[15,105] also prevents NF-κB effects *via* its known mechanism of transrepression.[106]

Upon radiation exposure of breast cancer tissue, HER-2 (Human Epidermal Growth Factor Receptor-2) exerts a pro-survival effect by NF-κB activation through Akt-mediated pro-survival pathways.[107] Interestingly, HER-2 itself is one of the genes activated by NF-κB upon radiation, suggesting a positive feedback loop between HER-2 and NF-κB.[108] Consequently, it is possible that FKBP52 could also positively regulate this loop *via* NF-κB offering a new therapeutic target for breast cancer treatment.

FKBP38

FKBP38 is a noncanonical FKBP family member that provides a scaffold platform to facilitate protein-protein interactions, in particular with anti-apoptotic factors.[109] Thus, the molecular interaction of FKBP38 with Bcl-2 contributes to tumorigenesis and chemoresistance.[110] Even though the PPIase domain of FKBP38 shows overall structural similarity to that shown by other immunophilins such as FKBP12, FKBP52 and Cyp40, it lacks the key residues required for FK506 binding and enzymatic activity.[111]

FKBP38 is involved in mTOR signal transduction pathway, favors tumor invasion and metastasis.[112] It is overexpressed in several types of human cancer cells and tumor tissues, including prostate, colon, breast, liver, lung, lymph node and stomach.[110,113–115] It is accepted that in cancer cells, FKBP38 blocks apoptosis mechanisms caused by calcium, staurosporine, cycloheximide, etoposide and UV radiation in a Bcl-2-dependent manner.[109,110]

FKBP38 may modulate the degradation of Bcl2 *via* proteasome by direct binding *via* its TPR domain to the S4 subunit of the 19S proteasome, which increases proteosomal activity in the membrane fractions.[116] Also, FKBP38 may modulate the cleavage of Bcl-2 by direct interaction with Bcl-2 and blocking the caspase-mediated cleavage pathway.[117] The stabilization of Bcl-2 by FKBP38 favors its accumulation, induces resistance to anticancer chemotherapy with cisplatin and paclitaxel and generates a poor prognosis.[118,119] Also, upregulation of Bcl-2 and Bcl-XL at transcriptional, translational and stability levels markedly protects neuroblastoma cells from apoptosis induced by cytotoxic agents.[120] Therefore, the FKBP38-dependent expression and maintenance of the function of Bcl-2 plays a pivotal role in the molecular mechanism to chemoresistance in cancer cells.

FKBPL

FKBPL/WisP39 was first identified as a gene down-regulated by radiation treatment,[32] which correlates with a radioresistant phenotype. Accordingly, the down-regulation of FKBPL affected cellular responses to radiotherapy, leading to increased DNA repair and cell survival.[121] FKBPL is also a key component of a heterocomplex involved in the post-translational stabilization of the cyclin-dependent kinase inhibitor, p21.[122] Along with Hsp90, it forms a trimeric complex with p21 preventing its proteasomal degradation, which initiates cell-cycle arrest following irradiation. The stabilization of p21 by the GTSE-1 (G2 and S phase-expressed-1) protein was also shown to be dependent on the FKBPL/WisP39•Hsp90 complexes, such that high level of GTSE-1 expression caused resistance to taxane chemotherapy modulating cell-cycle progression.[123]

As it was commented above, FKBPL shares the same properties of FKBP52 for the cytoplasmic retrotransport of GR.[35] It is also able to interact with the AR enhancing transcription[124] and the ER.[125] FKBPL/WisP39 modulates ER expression with an inverse correlation between FKBPL and ER levels,[125] leading to a decreased proliferation of breast cancer cell due to the inhibition of downstream signaling of ER-responsive genes. However, FKBPL/Wisp39 overexpression decreases ER phosphorylation *via* p21 stabilization, an event that has been linked to increased sensitivity to endocrine therapies.[126] Moreover, FKBPL/WisP39 affects the response to the ER antagonists tamoxifen and fulvestrant, since increased levels of expression of this immunophilin-like protein increases cell sensitivity to both drugs.[125] Consequently, FKBPL/Wisp39 is a biomarker to predict response to endocrine therapies.

Interestingly, FKBPL/WisP39 has also been related to angiogenesis since its secretion inhibits cell migration, tubule formation and angiogenesis.[127] Increased expression of FKBPL/WisP39 also leads growth inhibition of not only breast cancer cells,[125] but also myelocytic leukemia cells.[128] In the latter case, it does not induce significant apoptosis on leukemic cells, but it increases cell arrest at G_0/G_1 phase preventing cell proliferation.

Future Therapies with Selective Small Molecules

The developing of immunophilin ligands shows promising pharmacological perspectives in the near future. The ability to regulate the functions of a specific protein using cell-permeable small molecules is an unquestionable powerful method not only to study biological systems from the mechanistic perspective, but also a desired alternative to be used in therapeutic treatments. In this sense, Hsp90-binding immunophilins are novel targets that could offer new therapeutic opportunities in many fields, most likely in cancer therapy, as it is inferred from the previously discussed features of these proteins, but also in neurodegenerative diseases and other neurological disorders such as depression.[54] Following the isolation of rapamycin and CsA, it was FK506 the most used drug for the prevention of liver transplant rejection and since then, its use expanded rapidly into the transplantation of other organs (see Ref. [129 for a recent review). Strong attempts to synthesize new selective immunophilin ligands are in course of action.[130–134] Nonetheless, drug discovery has always been hampered because the failure to pharmacologically differentiate against the highly homologous members of the family, in particular for the case of FKBP51 and FKBP52. Most of the novel ligands are still unselective in this regard. However a recent publication described the properties of two new compounds named SAFit1 and SAFit2 that show selective antagonistic affinity by FKBP51[135] (K_i values equal to 4 and 6 nM, respectively) and >10,000 fold lower affinity for FKBP52.

Treatments with FK506 have been shown to inhibit the proliferation of prostate cancer cells and this fact was assigned to blockade of the enhancing effect of FKBP51 on the AR in these cells.[58,76] It has also been reported a physical association of FKBP51 with the Hsp90 bound kinase upstream of IκB, IKK *via* its IKK α subunit.[67,136] Consistent with this, the FKBP ligand rapamycin blocked IκB/NF-κB/mTOR signaling cascade[137]. Because the drug concentration

used is also able to inhibit FKBP52, it could also be possible that this effect on NF-κB signaling has actually been occurred due the inhibition of this stimulant immunophilin rather than prevention of the overall inhibitory action recently reported for FKBP51.[44] The development of selective drugs for each immunophilin, especially for FKB52, will help to answer this conundrum and to design eventual therapeutic approaches.

The developing of specific inhibitors for FKBP38 is even less prolific to date. Interestingly, the finding that cycloheximide, a well-known inhibitor of eukaryotic protein synthesis, also inhibits the PPIase activity of FKBP12, prompted the development of a derivative named N-(N′,N′-dimethyl-carboxamidomethyl)-cycloheximide that functions as a relatively specific inhibitor FKBP38.[138] Nonetheless, at the present its use is quite limited.

In view of the fact that FKBPL shows a wide variety of antitumor actions, this immunophilin is a quite promising pharmacologic target. Inasmuch as this divergent member of the FKBP subfamily lacks PPIase domain, the design of specific ligands is more difficult. Nonetheless, taking advantage of the antiantiangiogenic properties of FKBL, a peptide mimetic of FKBPL named ALM201 is currently being tested in clinical trials showing encouraging results to essentially correct FKBPL functional deficiencies in a number of diseases.[127,139]

Combined pharmacological approaches are also a feasible possibility. In this regard, treatments with CsA, a cycophilin-interacting drug, associated to other natural compounds such as sanglifehrin efficiently suppresses chemokine signalling and cell migration.[140] It has been proposed that the use of rapamycin along with metrothrexate and tacrolimus in patients with lymphoma is associated with a significantly decreased risk of disease progression,[141] although a recent clinical trial performed in children with acute lymphatic leukemia[142] also showed increased toxicity and disapproved the therapeutic combination of these drugs.

Probing pathways in response to specific inhibitors of immunophilins in cancer cells will become increasingly important during the next years. Development and characterization of novel small molecules able to target specifically members of the immunophilin family is an emerging field whose results will be seen in the very near future. This is expected especially for the molecular roles of FKBPL, FKBP51 and FKBP52 in cancer development and progression.

References

1. Schiene C, Fischer G. Enzymes that catalyse the restructuring of proteins. *Curr Opin Struct Biol* 2000;10:40–5.
2. Kang CB, Hong Y, Dhe-Paganon S, et al. FKBP family proteins: immunophilins with versatile biological functions. *Neurosignals* 2008;16:318–25.
3. Li H, Rao A, Hogan PG. Interaction of calcineurin with substrates and targeting proteins. *Trends Cell Biol* 2011;21:91–103.
4. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* 2014;15:155–62.
5. Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 1997;18:306–60.
6. Breiman A. Plant Hsp90 and its co-chaperones. *Curr Protein Pept Sci* 2014;15:232–44.
7. Kost SL, Smith DF, Sullivan WP, et al. Binding of heat shock proteins to the avian progesterone receptor. *Mol Cell Biol* 1989;9:3829–38.
8. Smith DF, Schowalter DB, Kost SL, et al. Reconstitution of progesterone receptor with heat shock proteins. *Mol Endocrinol* 1990;4:1704–11.
9. Baulieu EE, Binart N, Cadepond F, et al. Are receptor-associated nuclear proteins associated with the earliest effects of steroid hormones? *Symp Soc Exp Biol* 1990;44:3–20.
10. Pratt WB, Jolly DJ, Pratt DV, et al. A region in the steroid binding domain determines formation of the non-DNA-binding, 9 S glucocorticoid receptor complex. *J Biol Chem* 1988;263:267–73.
11. Renoir JM, Radanyi C, Faber LE, et al. The non-DNA-binding heterooligomeric form of mammalian steroid hormone receptors contains a hsp90-bound 59-kilodalton protein. *J Biol Chem* 1990;265:10740–5.
12. Galigniana MD, Echeverria PC, Erlejman AG, et al. Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore. *Nucleus* 2010;1:299–308.
13. Mazaira GI, Lagadari M, Erlejman AG, et al. The emerging role of TPR-domain immunophilins in the mechanism of action of steroid receptors. *Nucl Receptor Res* 2014;1:17.
14. Galigniana MD, Erlejman AG, Monte M, et al. The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events. *Mol Cell Biol* 2010;30:1285–98.
15. Wochnik GM, Ruegg J, Abel GA, et al. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. *J Biol Chem* 2005;280:4609–16.
16. Pekki A, Ylikomi T, Syvala H, et al. Progesterone receptor does not form oligomeric (8S), non-DNA-binding complex in intact cell nuclei. *J Cell Biochem* 1995;58:95–104.
17. Tuohimaa P, Pekki A, Blauer M, et al. Nuclear progesterone receptor is mainly heat shock protein 90-free in vivo. *Proc Natl Acad Sci USA* 1993;90:5848–52.
18. Renoir JM, Radanyi C, Jung-Testas I, et al. The nonactivated progesterone receptor is a nuclear heterooligomer. *J Biol Chem* 1990;265:14402–6.
19. Segnitz B, Gehring U. Subunit structure of the nonactivated human estrogen receptor. *Proc Natl Acad Sci USA* 1995;92:2179–83.
20. Pratt WB, Galigniana MD, Harrell JM, et al. Role of hsp90 and the hsp90-binding immunophilins in signalling protein movement. *Cell Signal* 2004;16:857–72.
21. Elbi C, Walker DA, Romero G, et al. Molecular chaperones function as steroid receptor nuclear mobility factors. *Proc Natl Acad Sci USA* 2004;101:2876–81.
22. Milgrom E. Activation of steroid-receptor complexed. New York: Academic Press, 1981.
23. Dahmer MK, Housley PR, Pratt WB. Effects of molybdate and endogenous inhibitors on steroid-receptor inactivation, transformation, and translocation. *Annu Rev Physiol* 1984;46:67–81.
24. Gasc JM, Renoir JM, Faber LE, et al. Nuclear localization of two steroid receptor-associated proteins, hsp90 and p59. *Exp Cell Res* 1990;186:362–7.
25. Echeverria PC, Mazaira G, Erlejman A, et al. 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* 2009;29:4788–97.
26. Grossmann C, Ruhs S, Langenbruch L, et al. Nuclear shuttling precedes dimerization in mineralocorticoid receptor signaling. *Chem Biol* 2012;19:742–51.
27. Presman DM, Ogara MF, Stortz M, et al. Live cell imaging unveils multiple domain requirements for in vivo dimerization of the glucocorticoid receptor. *PLoS Biol* 2014;12:e1001813
28. Barent RL, Nair SC, Carr DC, et al. Analysis of FKBP51/FKBP52 chimeras and mutants for Hsp90 binding and association with progesterone receptor complexes. *Mol Endocrinol* 1998;12:342–54.
29. Ratajczak T, Carrello A, Mark PJ, et al. The cyclophilin component of the unactivated estrogen receptor contains a tetratricopeptide repeat domain and shares identity with p59 (FKBP59). *J Biol Chem* 1993;268:13187–92.
30. Banerjee A, Periyasamy S, Wolf IM, et al. Control of glucocorticoid and progesterone receptor

subcellular localization by the ligand-binding domain is mediated by distinct interactions with tetratricopeptide repeat proteins. *Biochemistry* 2008;47:10471–80.
31. Hinds TD Jr., Sanchez ER. Protein phosphatase 5. *Int J Biochem Cell Biol* 2008;40:2358–62.
32. Robson TA, Lohrer H, Bailie JR, et al. Gene regulation by low-dose ionizing radiation in a normal human lung epithelial cell line. *Biochem Soc Trans* 1997;25:335–42.
33. Robson T, Joiner MC, Wilson GD, et al. A novel human stress response-related gene with a potential role in induced radioresistance. *Radiat Res* 1999;152:451–61.
34. McCalla DR, Allan RK. Effect of actinomycin D on euglena chloroplast formation. *Nature* 1964; 201:504–5.
35. McKeen HD, McAlpine K, Valentine A, et al. A novel FK506-like binding protein interacts with the glucocorticoid receptor and regulates steroid receptor signaling. *Endocrinology* 2008;149: 5724–34.
36. Taipale M, Krykbaeva I, Koeva M, et al. Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. *Cell* 2012;150:987–1001.
37. Galigniana MD, Harrell JM, O'Hagen HM, et al. Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. *J Biol Chem* 2004;279:22483–9.
38. Leo C, Chen JD. The SRC family of nuclear receptor coactivators. *Gene* 2000;245:1–11.
39. Werbajh S, Nojek I, Lanz R, et al. RAC-3 is a NF-kappa B coactivator. *FEBS Lett* 2000;485: 195–9.
40. Mussi P, Yu C, O'Malley BW, et al. Stimulation of steroid receptor coactivator-3 (SRC-3) gene overexpression by a positive regulatory loop of E2F1 and SRC-3. *Mol Endocrinol* 2006;20:3105–19.
41. Yan J, Yu CT, Ozen M, et al. Steroid receptor coactivator-3 and activator protein-1 coordinately regulate the transcription of components of the insulin-like growth factor/AKT signaling pathway. *Cancer Res* 2006;66:11039–46.
42. Arimura A, vn Peer M, Schroder AJ, et al. The transcriptional co-activator p/CIP (NCoA-3) is up-regulated by STAT6 and serves as a positive regulator of transcriptional activation by STAT6. *J Biol Chem* 2004;279:31105–12.
43. Colo GP, Rubio MF, Nojek IM, et al. The p160 nuclear receptor co-activator RAC3 exerts an anti-apoptotic role through a cytoplasmatic action. *Oncogene* 2008;27:2430–44.
44. Erlejman AG, De Leo SA, Mazaira GI, et al. Transcriptional activity is modulated by FK506-binding proteins FKBP51 and FKBP52: a role for peptidyl-prolyl isomerase activity. *J Biol Chem* 2014;289:26263–76.
45. Regan PL, Jacobs J, Wang G, et al. Hsp90 inhibition increases p53 expression and destabilizes MYCN and MYC in neuroblastoma. *Int J Oncol* 2011;38:105–12.
46. Whitesell L, Lin NU. HSP90 as a platform for the assembly of more effective cancer chemotherapy. *Biochim Biophys Acta* 2012;1823:756–66.
47. Trepel J, Mollapour M, Giaccone G, et al. Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer* 2010;10:537–49.
48. Denny WB, Prapapanich V, Smith DF, et al. Structure-function analysis of squirrel monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. *Endocrinology* 2005;146:3194–201.
49. Scammell JG, Denny WB, Valentine DL, et al. Overexpression of the FK506-binding immunophilin FKBP51 is the common cause of glucocorticoid resistance in three new world primates. *Gen Comp Endocrinol* 2001;124:152–65.
50. Reynolds PD, Roveda KP, Tucker JA, et al. Glucocorticoid-resistant B-lymphoblast cell line derived from the bolivian squirrel monkey (saimiri boliviensis boliviensis). *Lab Anim Sci* 1998; 48:364–70.
51. Hubler TR, Scammell JG. Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. *Cell Stress Chaperones* 2004;9:243–52.
52. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* 2001; 49:391–404.
53. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* 2009;34 Suppl 1: S186–95.
54. Galigniana NM, Ballmer LT, Toneatto J, et al. Regulation of the glucocorticoid response to stress-related disorders by the Hsp90-binding immunophilin FKBP51. *J Neurochem* 2012;122: 4–18.
55. Gallo LI, Lagadari M, Piwien-Pilipuk G, et al. 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* 2011; 286:30152–60.
56. Storer CL, Dickey CA, Galigniana MD, et al. FKBP51 and FKBP52 in signaling and disease. *Trends Endocrinol Metab* 2011; 22:481–90.
57. Kassi E, Moutsatsou P. Glucocorticoid receptor signaling and prostate cancer. *Cancer Lett* 2011; 302:1–10.
58. Periyasamy S, Hinds T, Jr., Shemshedini L, et al. FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin a. *Oncogene* 2010;29:1691–701.
59. Yong W, Yang Z, Periyasamy S, et al. Essential role for Co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology. *J Biol Chem* 2007;282:5026–36.
60. Knudsen KE, Penning TM. Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends Endocrinol Metab* 2010;21:315–24.
61. Estebanez-Perpina E, Moore JM, Mar E, et al. The molecular mechanisms of coactivator utilization in ligand-dependent transactivation by the androgen receptor. *J Biol Chem* 2005;280: 8060–8.
62. Sharifi N. Minireview: androgen metabolism in castration-resistant prostate cancer. *Mol Endocrinol* 2013;27:708–14.
63. Bonkhoff H, Berges R. From pathogenesis to prevention of castration resistant prostate cancer. *Prostate* 2010;70:100–12.
64. Linja MJ, Savinainen KJ, Saramaki OR, et al. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 2001;61:3550–5.
65. Egan A, Dong Y, Zhang H, et al. Castration-resistant prostate cancer: adaptive responses in the androgen axis. *Cancer Treat Rev* 2014;40: 426–33.
66. Kaikkonen S, Paakinaho V, Sutinen P, et al. Prostaglandin 15d-PGJ(2) inhibits androgen receptor signaling in prostate cancer cells. *Mol Endocrinol* 2013;27:212–23.
67. Jiang W, Cazacu S, Xiang C, et al. FK506 binding protein mediates glioma cell growth and sensitivity to rapamycin treatment by regulating NF-kappaB signaling pathway. *Neoplasia* 2008; 10:235–43.
68. Romano S, D'Angelillo A, Staibano S, et al. FK506-binding protein 51 is a possible novel tumoral marker. *Cell Death Dis* 2010;1:e55
69. Giraudier S, Chagraoui H, Komura E, et al. Overexpression of FKBP51 in idiopathic myelofibrosis regulates the growth factor independence of megakaryocyte progenitors. *Blood* 2002; 100:2932–40.
70. Avellino R, Romano S, Parasole R, et al. Rapamycin stimulates apoptosis of childhood acute lymphoblastic leukemia cells. *Blood* 2005;106: 1400–6.
71. Stechschulte LA, Sanchez ER. FKBP51-a selective modulator of glucocorticoid and androgen sensitivity. *Curr Opin Pharmacol* 2011;11:332–7.
72. Romano S, Staibano S, Greco A, et al. FK506 binding protein 51 positively regulates melanoma stemness and metastatic potential. *Cell Death Dis* 2013;4:e578
73. Hou J, Wang L. FKBP5 as a selection biomarker for gemcitabine and akt inhibitors in treatment of pancreatic cancer. *PLoS One* 2012;7:e36252
74. Solassol J, Mange A, Maudelonde T. FKBP family proteins as promising new biomarkers for cancer. *Curr Opin Pharmacol* 2011;11:320–5.
75. Pei H, Li L, Fridley BL, et al. FKBP51 affects cancer cell response to chemotherapy by negatively regulating akt. *Cancer Cell* 2009;16:259–66.
76. Ni L, Yang CS, Gioeli D, et al. FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. *Mol Cell Biol* 2010;30: 1243–53.
77. Romano S, D'Angelillo A, Pacelli R, et al. Role of FK506-binding protein 51 in the control of apoptosis of irradiated melanoma cells. *Cell Death Differ* 2010;17:145–57.
78. Mukaide H, Adachi Y, Taketani S, et al. FKBP51 expressed by both normal epithelial cells and adenocarcinoma of colon suppresses proliferation of colorectal adenocarcinoma. *Cancer Invest* 2008;26:385–90.
79. Callebaut I, Renoir JM, Lebeau MC, et al. An immunophilin that binds M(r) 90,000 heat shock protein: main structural features of a mammalian p59 protein. *Proc Natl Acad Sci USA* 1992;89:6270–4.
80. Wu B, Li P, Liu Y, et al. 3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex. *Proc Natl Acad Sci USA* 2004;101:8348–53.
81. Pirkl F, Buchner J. Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. *J Mol Biol* 2001;308:795–806.
82. Sinars CR, Cheung-Flynn J, Rimerman RA, et al. Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. *Proc Natl Acad Sci USA* 2003;100:868–73.

83. Riggs DL, Cox MB, Tardif HL, et al. Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling. *Mol Cell Biol* 2007;27:8658–69.
84. Ward BK, Mark PJ, Ingram DM, et al. Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer. *Breast Cancer Res Treat* 1999;58:267–80.
85. Shipp C, Watson K, Jones GL. Associations of HSP90 client proteins in human breast cancer. *Anticancer Res* 2011;31:2095–101.
86. Chen H, Yong W, Hinds TD, Jr., et al. Fkbp52 regulates androgen receptor transactivation activity and male urethra morphogenesis. *J Biol Chem* 2010;285:27776–84.
87. Yang Z, Wolf IM, Chen H, et al. FK506-binding protein 52 is essential to uterine reproductive physiology controlled by the progesterone receptor a isoform. *Mol Endocrinol* 2006;20:2682–94.
88. Tranguch S, Cheung-Flynn J, Daikoku T, et al. Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. *Proc Natl Acad Sci USA* 2005;102:14326–31.
89. Cheung-Flynn J, Prapapanich V, Cox MB, et al. Physiological role for the cochaperone FKBP52 in androgen receptor signaling. *Mol Endocrinol* 2005;19:1654–66.
90. Estebanez-Perpina E, Arnold LA, Nguyen P, et al. A surface on the androgen receptor that allosterically regulates coactivator binding. *Proc Natl Acad Sci USA* 2007;104:16074–9.
91. Grosdidier S, Fernandez-Recio J. Protein-protein docking and hot-spot prediction for drug discovery. *Curr Pharm Des* 2012;18:4607–18.
92. Grosdidier S, Carbo LR, Buzon V, et al. Allosteric conversation in the androgen receptor ligand-binding domain surfaces. *Mol Endocrinol* 2012;26:1078–90.
93. De Leon JT, Iwai A, Feau C, et al. Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. *Proc Natl Acad Sci USA* 2011;108:11878–83.
94. Ott M, Litzenburger UM, Rauschenbach KJ, et al. Suppression of TDO-mediated tryptophan catabolism in glioblastoma cells by a steroid-responsive FKBP52-dependent pathway. *Glia* 2015;63:78–90.
95. Opitz CA, Litzenburger UM, Sahm F, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 2011; 478:197–203.
96. Pilotte L, Larrieu P, Stroobant V, et al. Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. *Proc Natl Acad Sci USA* 2012;109:2497–502.
97. Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* 2006;25:6706–16.
98. Gilmore TD. The rel/NF-kappaB signal transduction pathway: introduction. *Oncogene* 1999; 18:6842–4.
99. Fan Y, Gupta N, Gelinas C. Molecular Basis of Oncogenesis by NF-kB: From a Bird's Eye View to Relevant Role in Cancer, ed. Georgetown, Texas, USA: Landes Bioscience, 2006.
100. Erlejman AG, De Leo SA, Mazaira GI, et al. NF-kappaB transcriptional activity is modulated by FK506-binding proteins FKBP51 and FKBP52: a ROLE FOR PEPTIDYL-PROLYL ISOMERASE ACTIVITY. *J Biol Chem* 2014;289: 26263–76.
101. Hinz M, Broemer M, Arslan SC, et al. Signal responsiveness of IkappaB kinases is determined by Cdc37-assisted transient interaction with Hsp90. *J Biol Chem* 2007;282:32311–9.
102. Galigniana MD, Radanyi C, Renoir JM, et al. Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus. *J Biol Chem* 2001;276:14884–9.
103. Mikenberg I, Widera D, Kaus A, et al. Transcription factor NF-kappaB is transported to the nucleus via cytoplasmic dynein/dynactin motor complex in hippocampal neurons. *PLoS One* 2007;2:e589
104. Mackenzie GG, Keen CL, Oteiza PI. Microtubules are required for NF-kappaB nuclear translocation in neuroblastoma IMR-32 cells: modulation by zinc. *J Neurochem* 2006;99:402–15.
105. Riggs DL, Cox MB, Cheung-Flynn J, et al. Functional specificity of co-chaperone interactions with Hsp90 client proteins. *Crit Rev Biochem Mol Biol* 2004;39:279–95.
106. Scheinman RI, Gualberto A, Jewell CM, et al. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 1995;15:943–53.
107. Guo G, Wang T, Gao Q, etal. Expression of ErbB2 enhances radiation-induced NF-kappaB activation. *Oncogene* 2004;23:535–45.
108. Cao N, Li S, Wang Z, et al. NF-kappaB-mediated HER2 overexpression in radiation-adaptive resistance. *Radiat Res* 2009;171:9–21.
109. Shirane M, Nakayama KI. Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. *Nat Cell Biol* 2003;5:28–37.
110. Choi BH, Yoon HS. FKBP38-Bcl-2 interaction: a novel link to chemoresistance. *Curr Opin Pharmacol* 2011;11:354–9.
111. Kang CB, Feng L, Chia J, et al. Molecular characterization of FK-506 binding protein 38 and its potential regulatory role on the anti-apoptotic protein Bcl-2. *Biochem Biophys Res Commun* 2005;337:30–8.
112. Bai X, Ma D, Liu A, et al. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science* 2007;318:977–80.
113. Azmi AS, Wang Z, Philip PA, et al. Emerging Bcl-2 inhibitors for the treatment of cancer. *Expert Opin Emerg Drugs* 2011;16:59–70.
114. Blaskovich MA, Yendluri V, Lawrence HR, et al. Lysophosphatidic acid acyltransferase beta regulates mTOR signaling. *PLoS One* 2013;8:e78632
115. Choi MS, Min SH, Jung H, et al. The essential role of FKBP38 in regulating phosphatase of regenerating liver 3 (PRL-3) protein stability. *Biochem Biophys Res Commun* 2011;406:305–9.
116. Nakagawa T, Shirane M, Iemura S, et al. Anchoring of the 26S proteasome to the organellar membrane by FKBP38. *Genes Cells* 2007; 12:709–19.
117. Choi BH, Feng L, Yoon HS. FKBP38 protects Bcl-2 from caspase-dependent degradation. *J Biol Chem* 2010;285:9770–9.
118. Sagawa Y, Fujitoh A, Nishi H, et al. Establishment of three cisplatin-resistant endometrial cancer cell lines using two methods of cisplatin exposure. *Tumour Biol* 2011;32:399–408.
119. Tabuchi Y, Matsuoka J, Gunduz M, et al. Resistance to paclitaxel therapy is related with Bcl-2 expression through an estrogen receptor mediated pathway in breast cancer. *Int J Oncol* 2009; 34:313–9.
120. Hadjidaniel MD, Reynolds CP. Antagonism of cytotoxic chemotherapy in neuroblastoma cell lines by 13-cis-retinoic acid is mediated by the antiapoptotic Bcl-2 family proteins. *Mol Cancer Ther* 2010;9:3164–74.
121. Robson T, Price ME, Moore ML, et al. Increased repair and cell survival in cells treated with DIR1 antisense oligonucleotides: implications for induced radioresistance. *Int J Radiat Biol* 2000; 76:617–23.
122. Jascur T, Brickner H, Salles-Passador I, et al. Regulation of p21(WAF1/CIP1) stability by WISp39, a Hsp90 binding TPR protein. *Mol Cell* 2005;17:237–49.
123. Bublik DR, Scolz M, Triolo G, et al. Human GTSE-1 regulates p21(CIP1/WAF1) stability conferring resistance to paclitaxel treatment. *J Biol Chem* 2010;285:5274–81.
124. Sunnotel O, Hiripi L, Lagan K, et al. Alterations in the steroid hormone receptor co-chaperone FKBPL are associated with male infertility: a case-control study. *Reprod Biol Endocrinol* 2010; 8:22
125. McKeen HD, Byrne C, Jithesh PV, et al. FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. *Cancer Res* 2010;70:1090–100.
126. Abukhdeir AM, Vitolo MI, Argani P, et al. Tamoxifen-stimulated growth of breast cancer due to p21 loss. *Proc Natl Acad Sci USA* 2008; 105:288–93.
127. Valentine A, O'Rourke M, Yakkundi A, et al. FKBPL and peptide derivatives: novel biological agents that inhibit angiogenesis by a CD44-dependent mechanism. *Clin Cancer Res* 2011;17: 1044–56.
128. Li YY, Liu LQ, Yang J, et al. [effect of WISp39 on proliferation, cell cycle and apoptosis of U937 cells]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2007;15:733–7.
129. Erlejman AG, Lagadari M, Galigniana MD. Hsp90-binding immunophilins as a potential new platform for drug treatment. *Future Med Chem* 2013;5:591–607.
130. Liu F, Wang YQ, Meng L, et al. FK506-binding protein 12 ligands: a patent review. *Expert Opin Ther Pat* 2013;23:1435–49.
131. Ashida T, Kikuchi T. Estimation of relative binding free energy based on a free energy variational principle for the FKBP-ligand system. *J Comput Aided Mol Des* 2013;27:479–90.
132. Wang Y, Kirschner A, Fabian AK, et al. Increasing the efficiency of ligands for FK506-binding protein 51 by conformational control. *J Med Chem* 2013;56:3922–35.
133. Galat A. Functional diversity and pharmacological profiles of the FKBPs and their complexes with small natural ligands. *Cell Mol Life Sci* 2013;70:3243–75.
134. Schmidt MV, Paez-Pereda M, Holsboer F, et al. The prospect of FKBP51 as a drug target. *ChemMedChem* 2012;7:1351–9.
135. Gaali S, Kirschner A, Cuboni S, et al. Selective inhibitors of the FK506-binding protein 51 by induced fit. *Nat Chem Biol* 2015;11:33–7.
136. Bouwmeester T, Bauch A, Ruffner H, et al. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. *Nat Cell Biol* 2004;6:97–105.

137. Giordano A, Avellino R, Ferraro P, et al. Rapamycin antagonizes NF-kappaB nuclear translocation activated by TNF-alpha in primary vascular smooth muscle cells and enhances apoptosis. *Am J Physiol Heart Circ Physiol* 2006; 290:H2459–65.
138. Edlich F, Weiwad M, Wildemann D, et al. The specific FKBP38 inhibitor N-(N',N'-dimethylcarboxamidomethyl)cycloheximide has potent neuroprotective and neurotrophic properties in brain ischemia. *J Biol Chem* 2006;281:14961–70.
139. Robson T, James IF. The therapeutic and diagnostic potential of FKBPL; a novel anticancer protein. *Drug Discov Today* 2012;17:544–8.
140. Immecke SN, Baal N, Wilhelm J, et al. The cyclophilin-binding agent sanglifehrin a is a dendritic cell chemokine and migration inhibitor. *PLoS One* 2011;6:e18406
141. Armand P, Gannamaneni S, Kim HT, et al. Improved survival in lymphoma patients receiving sirolimus for graft-versus-host disease prophylaxis after allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning. *J Clin Oncol* 2008;26:5767–74.
142. Pulsipher MA, Langholz B, Wall DA, et al. The addition of sirolimus to tacrolimus/methotrexate GVHD prophylaxis in children with ALL: a phase 3 children's oncology group/pediatric blood and marrow transplant consortium trial. *Blood* 2014;123:2017–25.
143. Lupski JR, de Oca-Luna RM, Slaugenhaupt S, et al. DNA duplication associated with Charcot-Marie-tooth disease type 1A. *Cell* 1991;66:219–32.
144. Wang T, Li BY, Danielson PD, et al. The immunophilin FKBP12 functions as a common inhibitor of the TGF beta family type I receptors. *Cell* 1996;86:435–44.
145. Cameron AM, Steiner JP, Roskams AJ, et al. Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca2+ flux. *Cell* 1995;83:463–72.
146. Jayaraman T, Brillantes AM, Timerman AP, et al. FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J Biol Chem* 1992;267:9474–7.
147. Gallo LI, Ghini AA, Piwien Pilipuk G, et al. 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* 2007;46:14044–57.
148. Cox MB, Smith DF. Functions of the Hsp90-Bindign FKBP Immunophilins. In: Blatch GL, ed. The Networking of Chaperones by Cochaperones: Eurekah.com, Landes Bioscience, Austin, Texas, USA, 2006.
149. Erlejman AG, Lagadari M, Harris DC, et al. Molecular chaperone activity and biological regulatory actions of the TPR-domain immunophilins FKBP51 and FKBP52. *Curr Protein Pept Sci* 2014;15:205-15
150. Quintá HR, Maschi D, Gomez-Sanchez C, Piwien-Pilipuk G, Galigniana MD. Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth. *J Neurochem* 2010;115:716–34.
151. Sivils JC, Storer CL, Galigniana MD, et al. Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52). *Curr Opin Pharmacol* 2011;11:314–9.
152. Sanokawa-Akakura R, Dai H, Akakura S, et al. A novel role for the immunophilin FKBP52 in copper transport. *J Biol Chem* 2004;279:27845–8.
153. Presman DM, Alvarez LD, Levi V, et al. Insights on glucocorticoid receptor activity modulation through the binding of rigid steroids. *PLoS One* 2010;5:e13279