The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBPL

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Abstract

FKBPs (FK506-binding proteins) have long been recognized as key regulators of the response to immunosuppressant drugs and as co-chaperones of steroid receptor complexes. More recently, evidence has emerged suggesting that this diverse protein family may also represent cancer biomarkers owing to their roles in cancer progression and response to treatment. FKBPL (FKBP-like) is a novel FKB with roles in GR (glucocorticoid receptor), AR (androgen receptor) and ER (oestrogen receptor) signalling. FKBPL binds Hsp90 (heat-shock protein 90) and modulates translocation, transcriptional activation and phosphorylation of these steroid receptors. It has been proposed as a novel prognostic and predictive biomarker, where high levels predict for increased recurrence-free survival in breast cancer patients and enhanced sensitivity to endocrine therapy. Since this protein family has roles in a plethora of signalling pathways, its members represent novel prognostic markers and therapeutic targets for cancer diagnosis and treatment.

Introduction

The complexity of cancer makes it essential to identify novel predictive and prognostic biomarkers. Accurate predictions of response to cancer therapy will enable treatments to be tailored to each individual patient, thereby preventing overtreatment with potentially harmful agents that provide no therapeutic benefit. Additionally, prognostic markers give valuable information on overall patient survival and the probability of disease recurrence. This review focuses on FKBPs (FK506-binding proteins), since these proteins are emerging as important regulators of cancer progression and treatment response, as well as having a role in tumour invasion and metastasis.

FKBPs are members of the immunophilin protein superfamily characterized by the presence of PPIase (peptidylprolyl cis–trans isomerase) and TPR (tetratricopeptide repeat) domains within their protein structure. The PPIase domain is required to accelerate isomerization of peptidylprolyl bonds during protein folding in order to prevent degradation of partially folded proteins [1], while the TPR domain is required for binding to Hsp90 (heat-shock protein 90) [2,3]. However, FKBPs were first identified in the binding of the immunosuppressant molecule tacrolimus (originally designated FK506), which inhibits calcineurin signalling, blocking T-lymphocyte signal transduction. In addition to binding the immunosuppressant drug FK506 [4], they also have well-known roles as co-chaperones in large steroid receptor complexes, where they facilitate the maintenance of receptor stability in the absence of a ligand [5]. New members of this family have emerged over the last few years, most notably FKBPL (FKBP-like), which has been shown to be an important intracellular signalling molecule that may also be useful as a prognostic and predictive biomarker.

Isolation and characterization of the novel FKBPL protein FKBP

FKBPL (DIR1/WISp39) is located on chromosome 6p21.3 and was initially identified as a gene down-regulated by...
low-dose radiation in the 0.05–1 Gy range [6]. Subsequent analysis revealed that FKBPL was a divergent member of this group with shared homology, mostly in the C-terminal TPR domain, important for interactions with Hsp90. FKBPL shows low homology over the PPIase domain and lacks critical residues that are required for enzymatic activity [7]. Further studies demonstrated that FKBPL is down-regulated by low-dose ionizing radiation, which correlated with a radioresistant phenotype; in this respect down-regulation of FKBPL levels using antisense oligonucleotides affected cellular responses to radiotherapy, leading to increased DNA repair and cell survival [8]. More recent studies have determined that FKBPL is an important component of a complex involved in the post-translational stabilization of the CDK (cyclin-dependent kinase) inhibitor p21 [9]. In that study, FKBPL was shown to recruit Hsp90 to form a trimeric complex with newly synthesized p21 in order to prevent its proteasomal degradation, thus initiating cell-cycle arrest following irradiation. More recently, p21 stabilization by the GTSE-1 (G2 and S phase-expressed-1) protein was shown to be dependent on the Hsp90–FKBPL complex; high-level expression of GTSE-1, concomitant with p21 regulation, caused resistance to taxane chemotherapy [10]. Overall, these studies demonstrate that FKBPL plays an important role modulating cell-cycle progression, with implications for cell survival following stress induced by both radiotherapy and chemotherapy.

FKBPs and steroid receptor signalling

FKBPs are known to be important in steroid receptor complexes. Primarily, they exist as a component of a multi-chaperone complex along with Hsp90 and a variety of other co-chaperones to maintain steroid receptors in a conformation that enables rapid ligand binding and transactivation of steroid-receptor-responsive genes. More recently, it has become evident that FKBPs can have a major impact on cancer initiation, progression and therapy through perturbation of steroid receptor signalling.

GR (glucocorticoid receptor)

The GR, in the unliganded form is mainly a cytoplasmic protein that is transported to the nucleus, following hormone binding, to modify transcriptional activity of genes that contain glucocorticoid response elements within their promoter [11]. Previously, the PPIase domain of FKBP52 was identified as an interaction domain for the dynein motor protein [12] and when targeted with FKBP52-specific antibodies, GR translocation into and out of the nucleus was hindered [13]. More recently, FKBP51 and FKBP52 have been linked with rapid GR translocation specifically [14,15]. FKBPL also appears to have a similar role in hsp90 complexes associated with GR. In prostate cancer cell lines, FKBPL interacts with GR and Hsp90 and affects GR transactivation [16]. Furthermore, in cancer cells, FKBPL levels correlated with GR levels, but inversely correlated in transformed normal cells, suggesting that, like other FKBPs, the effects of FKBPL are cell-type-dependent. Furthermore, FKBPL was shown to interact with the dynein motor protein dynamitin and translocate to the nucleus following treatment with the GR ligand dexamethasone, suggesting that FKBPL is not only involved in the modulation of GR levels, but is also important for transportation of the receptor to the nucleus via microtubules. The ability of these FKBPs to modify the localization of GR could have implications for cancer, and a recent study has established that FKBP51 is involved in the suppression of cell proliferation in colorectal adenocarcinoma [17].

AR (androgen receptor)

The inhibition of AR signalling through androgen deprivation-based therapies has been a major approach in the treatment of prostate cancer. The link between FKBPs and AR signalling has long been established. FKBP51 was identified as an androgen-regulated gene [18] that increases transcriptional activity of the AR [19]. More recently, FKBP51 has been linked with promoting the assembly of superchaperone complexes in androgen-independent prostate cancer, thereby enhancing cell growth at low androgen concentrations [20]. FKBP52 has been shown to be up-regulated in prostate cancer [21], while FKBP52-null male mice displayed infertility and reduced AR activity [22,23]. FKBPL has also been linked with the AR, with particular reference to male infertility. It is located in the region of chromosome 6 that is linked with azoosperma in the Japanese
High FKBPL expression correlates with improved patient survival and increased sensitivity to endocrine therapy through a modulation of the p21 and ER signalling pathways. High FKBPL levels lead to p21 stabilization through the FKBPL/Hsp90 complex, causing impaired CDK signalling and inhibition of both cell cycle progression and ER phosphorylation on Ser118. In addition, high FKBPL causes decreased levels of ER, thereby reducing the number of ER molecules available for Ser118 phosphorylation. The combined effect of inhibiting both cell cycle progression and activation of ER-responsive genes through phosphorylation would cause reduced tumour cell growth and increased sensitivity to endocrine therapy drugs.

The relationship between FKBPs and the ER has been well characterized. FKBP52 is an oestrogen-inducible gene [26] that is up-regulated in breast cancer [27,28]. More recently, FKBP52 was shown to be specifically methylated in ER-negative, but not ER-positive, breast cancer cell lines, suggesting that FKBP52 may be involved in regulating ER expression [29]. A recent study has also determined that FKBPL plays a significant role in ER signalling in breast cancer cells with implications for cancer treatment [30]. This study found that FKBPL is also an oestrogen-responsive gene that interacts with ER within the Hsp90 chaperone complex. It is well established that ER levels are tightly regulated to allow an appropriate response to oestrogen [31]. FKBPL was found to modulate ER expression, with an inverse correlation between FKBPL and ER levels [30]. A consequence of FKBPL-mediated ER modulation was a decrease in breast cancer cell proliferation due to the inhibition of downstream signalling to ER-responsive genes. Furthermore, FKBPL overexpression leads to decreased ER phosphorylation on Ser118, possibly via p21 stabilization, which has been previously linked with increased sensitivity to endocrine therapy [32].

ER (oestrogen receptor)

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FKBPs as cancer biomarkers

Recent studies have established roles for FKBPs as both predictive and prognostic cancer biomarkers. Each FKB displays a unique function, independent of PPIase activity, with expression levels often influencing cellular responses to cancer therapies, although these responses are dependent on cancer type. Erlotinib is an EGFR (epidermal growth factor receptor) tyrosine kinase inhibitor, which is highly successful in treating human GBM (glioblastoma...
Table 1 | Analysis of FKBPL protein expression levels with clinicopathological parameters in a breast tumour patient cohort (n = 498)

Values in parentheses are percentages. P values were calculated using χ² test and Pearson’s correlation coefficient.

<table>
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<th>FKBPL 1 + (n = 108)</th>
<th>FKBPL 2 + (n = 262)</th>
<th>FKBPL 3 + (n = 107)</th>
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multiforme). FKBP14 has been associated with EGFR signalling and conveying an erlotinib-resistant phenotype in GBM [33]. FKBP51 expression is increased in glioma cells compared with normal tissue, and these increased levels enhanced cell growth, causing resistance to rapamycin [34]. However, FKBP51 must regulate cell survival through multiple pathways, since decreased expression can lead to resistance to topoisomerase inhibitors and microtubule stabilizers [35] and radioreistance in melanoma cells [36]. FKBP65 has been implicated in the initiation of colorectal carcinogenesis, with high expression in lung cancer and sarcomas, but decreased expression in ovarian cancer [37]. Increased levels of FKBP1a and FKBP8 have been shown to up-regulate Syndecan 1, a protein that increases cell adhesion to the extracellular matrix and inhibits the pro-invasive MMP-9 (matrix metalloproteinase-9), thereby demonstrating that adhesion and matrix remodelling pathways mediate the anti-invasive and anti-metastatic effects of these FKBP[38]. In childhood astrocytoma (high grade), the FKBPL2/EGFR/HIF-2α (hypoxia-inducible 2α) pathway has been implicated in the promotion of angiogenesis, and blocking this pathway with immunosuppressants such as rapamycin and FK506 may have implications for treatment of this disease [39].

FKBPL: a novel prognostic/predictive biomarker in breast cancer?

FKBPL clearly has a role in Hsp90 chaperone complexes associated with ER [30]. One of the major factors in the development of resistance to endocrine therapies is the ability of breast cancer cells to overcome the low-oestrogen conditions imposed by these drugs. However, high FKBPL levels have been shown to re-sensitize cells to this low-oestrogen environment, significantly inhibiting growth [30], suggesting that FKBPL might be predictive of response to aromatase inhibitors. Furthermore, FKBPL has an important influence over response to the ER antagonists tamoxifen and fulvestrant, where high levels of FKBPL increase cellular sensitivity to these drugs [30]. Therefore FKBPL could represent a novel biomarker that predicts response to a range of endocrine therapies. Furthermore, as with FKBPL2, FKBPL has also been implicated in controlling angiogenesis. It has been identified as a naturally
secreted, anti-angiogenic protein that inhibits migration, tubule formation and angiogenesis through a CD44-mediated pathway, but not proliferation of endothelial cells [40]. Therefore, since it has neither the broad spectrum anti-proliferative properties of clinically used anti-angiogenics nor the serious side effects these induce, its peptide derivatives are being developed as potential alternatives to current anti-angiogenic therapies [40]. In addition, FKBP1 is suggested as a potent diagnostic biomarker for increased patient survival. Increased expression of FKBP1 leads to both leukemic cell [41] and breast cancer cell [30] growth inhibition. Moreover, loss of the region of chromosome 6 on which FKBP1 is located has been shown to occur more frequently in patients presenting with cancer recurrence within 5 years of diagnosis [42]. Analysis of publicly available microarray datasets has revealed that there is a highly significant correlation between FKBP1 mRNA levels and improved survival of breast cancer patients \(P = 0.0039\) [30]. More recently, screening of a tissue microarray cohort [43] revealed that FKBP1 is widely expressed in breast cancer tissue, and increased levels of FKBP1 are associated with smaller, low-grade tumours (Table 1). Survival analysis demonstrated a statistically significant separation in survival curves, where high FKBP1 was associated with prolonged recurrence-free survival \(P = 0.005\) (Figure 1).

Overall, FKBP1 exhibits the multifunctional nature of other FKBP proteins and may represent an important protein for predicting patient survival outcomes and treatment response through a modulation of p21 and ER signalling pathways (Figure 2).

**Conclusion**

FKBPs have been well characterized in steroid receptor signalling and are now emerging as novel cancer biomarkers. The diversity of these TPR-containing proteins along with their versatile biological functions in a host of cell signalling pathways also provides further opportunities for therapeutic intervention. Further analysis of these proteins along with the identification of additional family members will be important for understanding their function in a host of disease processes such as cancer.

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**References**


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