The therapeutic and diagnostic potential of FKBPL; a novel anticancer protein

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The immunophilin family of proteins has a vast number of roles regulating a variety of biological processes through protein–protein interactions. A relatively new and divergent member of this family, FK506-binding protein like (FKBPL), is emerging as a key player in the DNA damage response, steroid receptor signalling and more recently, control of tumour growth where it regulates response to endocrine therapy in addition to acting as a novel antiangiogenic protein. As a new therapeutic peptide based on FKBPL approaches clinical trials, this article highlights a unique approach to targeting tumours that are resistant to current antiangiogenic therapies and supports the role of FKBPL as a novel prognostic and predictive biomarker, distinct from its other family members.

FK506-binding proteins (FKBPs) are members of the immunophilin family; other members include the cyclophilins and parvulins [1,2]. The term immunophilin, relates to the ability of this protein family to bind the immunosuppressive drugs, FK506 and rapamycin, through their peptidyl prolyl cis/trans isomerase (PPIase) domain. This interaction leads to inhibition of calcineurin or mammalian target of rapamycin (mTOR) signalling (by FK506 and rapamycin, respectively) which reduces T cell activation and mediates immune suppression; although inhibition of PPIase activity is not involved. Family members are distinguished by their molecular weight and domain structure. FKBP12, a smaller member, consists mainly of the PPIase domain and interacts strongly with immunosuppressive drugs while the larger, non-canonical FKBP members, such as FKBP38, have a PPIase domain that does not bind immunophilin ligands [3]. The larger proteins, FKBPS1 and S2, have duplicated PPIase domains, one of which can bind ligand, in addition to tetra-trico-peptide repeat (TPR) domains, which facilitate protein–protein interactions and a C-terminal calmodulin-binding domain [4]. In addition to their roles in immunosuppression, FKBPs have a wider variety of biological functions, such as regulatory or stabilizing components of multi-protein complexes integral to cell function and cell cycle control [3,4]. Immunophilins are most widely known for their biological role as co-chaperones within steroid hormone receptor complexes where they associate with the heat shock protein, Hsp90, [5] through their TPR domain and regulate steroid receptor signalling [6]. In 1997, a novel but clearly divergent member of this family, FK506-binding protein like (FKBPL), was identified [7]. It shares homology with the FKBP family (in particular FKBPS2/51 and cyclophilin 40) mostly in the C-terminal TPR domain [8], but lacks the crucial residues within a weakly homologous PPIase domain that are required for enzymatic activity [9]. The sequence of FKBPL and relationship to other FKBPs is shown in Fig. 1. Recent research is now revealing the functional characteristics of FKBPL, highlighting its antitumour activity by several diverse mechanisms.

Discovery of the FKBPL gene and its role in radioresistance
FKBPL was discovered from a screen to identify genes responsible for induced radioresistance [7]. A clone, originally designated ‘8.6’; then subsequently ‘downregulated by ionizing radiation’ (DIR1) [8], was finally renamed FKBPL, following the identification of its FKBP-like structure. Knockdown of FKBPL using antisense oligonucleotides, resulted in cell cycle checkpoint activation, and enhanced DNA repair and
clonogenic survival following exposure to ionizing radiation \[8,10\]. Together the data suggested a role in radioresistance, a phenomenon not previously noted at that time for other immunophilins. More recently, it has been shown that high (rather than low) levels of FKBP51 lead to inhibition of apoptosis and increasing radioresistance\[11\].

FKBPL was also pulled out of a screen to identify p21-associated proteins \[12\] where it was described as WISP39 (WAF-1/CIP1 stabilizing protein 39). It was discovered that FKBPL/WISP39 has an important role in a novel multiprotein complex, crucial to a G2 cell cycle checkpoint following high dose radiation stress. FKBPL binds to newly synthesized p21, in a complex with Hsp90, increasing p21 stability by preventing its proteasomal degradation, enhancing the G2 arrest. Jascur et al. suggested that targeting FKBPL in tumours, for which p21 overexpression confers a pro-survival growth advantage and/or chemoresistance/radioresistance, could be therapeutically beneficial. Although our own data \[8,10\] suggest that FKBPL is downregulated by radiation, which would be inconsistent with the stabilization of p21 observed by Jascur et al. \[12\], this could be due to tissue-specific differences within the cell lines used or because of a dependency of cell lines on a particular pathway. Indeed, in some instances knockdown of p21 results in a decrease in arrested cells and an increase in survival as described by Chu et al. \[13\]; this would be consistent with our own data where FKBPL knockdown, and presumably reduced p21, confers resistance to radiation \[8,10\].

The available data \[7,8,10,12\] suggested that the level of FKBPL within a tumour could indeed be important for the control of growth and response to therapy. In this respect, FKBP51 regulates AKT phosphorylation through a scaffolding mechanism and, as a result, can influence response to a variety of antineoplastic agents \[14\]. The role of FKBPL in mediating resistance and/or sensitivity to chemotherapeutic agents is currently the focus of investigations in our own laboratory. However, others have demonstrated that the association of the FKBPL/Hsp90/p21 complex with high levels of GSE-1 (G2 and S phase expressed protein 1) increased p21 stabilization and causes resistance to taxane chemotherapy \[15\], supporting a role for FKBPL in pathways associated with chemoresistance. Furthermore, threonine-193 of FKBPL is phosphorylated in response to DNA damage on consensus sites recognized by ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) \[16\] further highlighting a role for FKBPL in response to therapy.

The role of FKBPL in Hsp90 chaperone complexes

The role of FKBPL within Hsp90 chaperone complexes, first described by Jascur et al. \[12\], was not surprising given its homology across the TPR domains to large immunophilins, and the importance of the TPR domain for interaction with Hsp90. The main role of immunophilins within these complexes is to maintain the steroid
FKBPL as a secreted antiangiogenic protein

Full length recombinant FKBPL (rFKBPL) is a highly potent antiangiogenic protein, inhibiting endothelial cell migration and tube formation, in addition to microvessel formation from rat aortic rings ex vivo or within murine sponges in vivo [27]. In mouse xenograft studies twice weekly intratumoral injections of an FKBPL expression construct caused long-lasting inhibition of tumour growth and extensive central tumour necrosis, similar to cavitation reported in clinical trials of angiogenesis inhibitors [28].

The N-terminal region of FKBPL protein is responsible for the antiangiogenic activity (Fig. 1), the sequence is unique, with no homology to other FKBP s or other proteins. Peptides from this region are at least equipotent to full length FKBPL in endothelial cell migration assays. Detailed characterisation of a 24-residue peptide (AD-01) comprising amino acids 34–58 of FKBPL showed inhibition of endothelial cell migration, tubule formation and vessel sprouting from aortic rings with potencies in the picomolar range [27]. In xenograft experiments, daily systemic delivery of AD-01 inhibited blood vessel development and reduced tumour growth.

A growing body of evidence [27] suggests that these antiangiogenic effects are initiated from outside of endothelial cells, because both rFKBPL and AD-01 applied extracellularly are potent in all the models of angiogenesis tested. Consistent with this, endogenous FKBPL is secreted from several cell types including endothelial cells and tumour cells [27]. The mechanism of action is dependent on CD44; AD01 is inactive in cells that

Table 1: Comparison of ALM201 with marketed antiangiogenic drugs

<table>
<thead>
<tr>
<th>Assay</th>
<th>ALM201 (nM)</th>
<th>Bevacizumab</th>
<th>Sunitinib</th>
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<tbody>
<tr>
<td>Wound scrape</td>
<td>0.009</td>
<td>59</td>
<td>8.9</td>
</tr>
<tr>
<td>Tubule formation</td>
<td>0.082</td>
<td>2400</td>
<td>4.4</td>
</tr>
<tr>
<td>Proliferation</td>
<td>No effect at 10 000</td>
<td>158</td>
<td>1300</td>
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*Data are from three independent experiments.

**Humanized monoclonal antibody that inhibits VEGF-A.

***Small molecule, multi-targeted receptor tyrosine kinase inhibitor, which targets VEGFR, PDGFR, C-kit and Flt-3.

* The FKBPL derivative ALM201 was compared with bevacizumab and sunitinib in a range of angiogenic assays in vitro.
do not express endogenous CD44 and in cells where CD44 is downregulated with siRNA [27]. The role of CD44 is currently under investigation with respect to mediating peptide uptake and the subsequent antiangiogenic signalling.

Development of FKBPL peptide derivatives for clinical trials

The novel mechanism of action and the high potency of AD01 derived from FKBPL make peptides based on AD01 attractive candidates for development as new antiangiogenic drugs. Structure/activity and peptide stability studies using AD01 as the starting point led to the selection of ALM201*, a 23-residue peptide comprising amino acids 35–58 of the FKBPL sequence, as the drug development candidate. ALM201 is much more potent than marketed antiangiogenics in several models and has no effect on cell proliferation (Table 1). Preclinical activities with ALM201 including formulation and toxicology have been completed and the compound is approaching Phase I clinical trials. The clear differentiation from other antiangiogenics in terms of potency, lack of cytotoxicity and novel mechanism of action makes ALM201 an attractive drug candidate with potential roles in many solid tumours and in patients where anti-VEGF (vascular endothelial growth factor) therapy is ineffective or in tumours that develop resistance to current VEGF-targeted therapies.

Concluding remarks

The roles of FKBPL are summarised in Fig. 2. FKBPL is clearly a divergent immunophilin with distinct and important functions in cancer. Emerging evidence is also highlighting activity in other disease states, such as azoospermia [9] and Alzheimer’s, where it appears to have neuroprotective properties [29]. Drug development based on FKBPL is being actively pursued in the angiogenesis area, where a clinical candidate is approaching Phase I cancer trials. In addition to its therapeutic potential, the utility of FKBPL as a cancer biomarker, although still in the early stages, might facilitate more tailored cancer therapies with particular attention needed with

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respect to understanding its role in influencing tumour sensitivity to chemotherapeutic agents. The roles of FKBP family in other disease states are only just emerging, highlighting that it might take another 10 years before the functional and therapeutic significance of this protein is fully realised.

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