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IRF4 in multiple myeloma—Biology, disease and therapeutic target

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ABSTRACT

Multiple Myeloma (MM) is an incurable hematologic malignancy characterized by abnormal proliferation of plasma cells. Interferon Regulatory Factor 4 (IRF4), a member of the interferon regulatory family of transcription factors, is central to the genesis of MM. IRF4 is highly expressed in B cells and plasma cells where it plays essential roles in controlling B cell to plasma cell differentiation and immunoglobulin class switching. Overexpression of IRF4 is found in MM patients' derived cells, often as a result of activating mutations or translocations, where it is required for their survival. In this review, we first describe the roles of IRF4 in B cells and plasma cells and then analyse the subversion of the IRF4 transcriptional network in MM. Moreover, we discuss current therapies for MM as well as direct targeting of IRF4 as a potential new therapeutic strategy.

1. Introduction

Multiple Myeloma (MM) is an aggressive and incurable cancer characterized by the clonal proliferation of bone marrow plasma cells. MM diagnosis follows the appearance of end-organ damage known as the CRAB criteria (increased calcium level, renal dysfunction, anaemia, and destructive bone lesions) but can also be diagnosed in presence of at least one myeloma defining event or MED (bone marrow plasma cells greater than or equal to 60%; serum free light chain ratio greater than or equal to 100 provided involved FLC level is at least 100 mg/L; more than one focal lesion on magnetic resonance imaging that is at least 5 mm or greater in size) [1]. MM represents approximately 2% of all cancers and about 10% of all hematologic malignancies [2] with a rising incidence estimated to be 6–10 cases per 100,000 persons per year. In the UK alone 5540 people were diagnosed and 3079 deaths were reported in 2016. The median age of patients at the time of diagnosis is about 65 years [2]. MM is considered a multistep disease since almost all patients with MM are characterized by an asymptomatic pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS) and some patients by an intermediate asymptomatic but more advanced pre-malignant stage called smouldering multiple myeloma (SMM) [2,3].

Therapies used in the treatment of MM include initial therapy, autologous stem cell transplantation (when possible), consolidation/maintenance therapy, and treatment of relapse [4]. The most common regimes for MM initial therapy consist of a combination of drugs including immunomodulatory drugs (IMiD) (thalomide, lenalidomide), corticosteroids (dexamethasone) and proteasome inhibitors (PI) (bortezomib). Current treatments have dramatically improved the median overall survival of patients, however MM usually relapses with patients refractory to both IMiDs and PIs. In the last few years, treatment of relapsed refractory MM improved because of the introduction of pomalidomide, another immune-modifying drug, monoclonal antibodies daratumumab and elotuzumab, the histone deacetylase inhibitor panobinostat and new-generation proteasome inhibitors carfilzomib and ixazomib [4]. However with a median duration between MM diagnosis and relapse of 3.1 years and a median overall survival following relapse of 13 months [5], there is a clear need for new treatments to overcome the dismal survival rates of MM.

Interferon Regulatory Factor 4 (IRF4) is a transcription factor belonging to the interferon regulatory factor (IRF) family. IRFs are transcription factors playing a critical role in the regulation of immune responses, immune cell development, cell growth regulation and metabolism [6]. IRF4 is a critical regulator of the immune system and it is essential for PC differentiation [7,8]. IRF4 has also emerged as the master regulator of an aberrant and malignancy-specific gene expression programme in MM, where it is found to be overexpressed often as a result of activating mutations or translocations [9,10]. Knockdown experiments of IRF4 have shown a dramatic decrease in the viability of MM cells [10]. Enforced expression of miR-125b-5p, a miRNAs predicted to target the 3′ UTR of IRF4 mRNA, inhibits the growth and survival of MM cell lines [11]. Yet IRF4 has not been the direct target of therapeutic drug discovery programmes.

Here we describe the role of IRF4 during normal PC differentiation,

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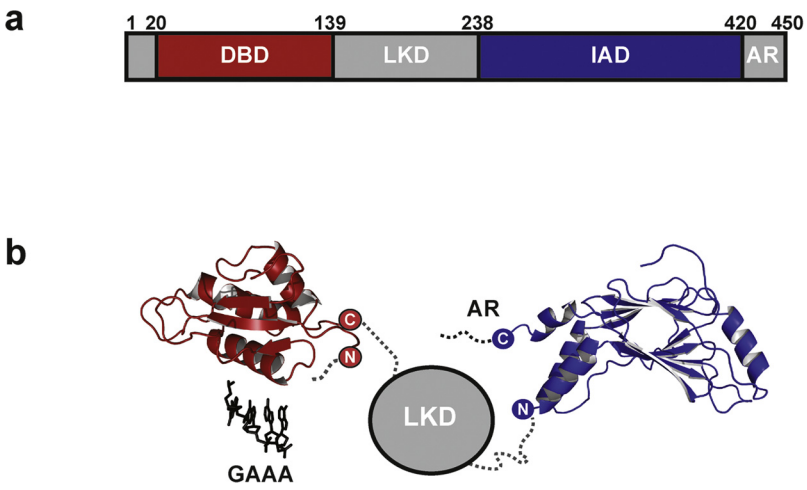

Fig. 1. Overall structure of IRF4. (a) Schematic representation showing the domain arrangement of IRF4: DNA binding domain (DBD, red), linker domain (LKD), Interferon Activating Domain (IAD, blue), auto inhibitory region (AR). **(b)** Cartoon representation of the crystal structure of the IRF4 DBD bound to GAAA consensus motif [12] and IAD (PDB: 5BVI). The LKD domain, which is thought to be folded into a domain structure, interacts with both DBD and IAD domains [6]. The AR domain is flexible and does not interact with either IAD or DBD domains [6]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

the mechanism of IRF4-driven deregulation of transcriptional activity in MM and we discuss the value of new therapeutic avenues to treat MM, including the direct targeting of IRF4.

2. IRF4 structure and transcriptional activity

IRF4 is characterized by an N-terminal tryptophan pentad repeat DNA-binding domain (DBD) connected to a C-terminal interferon activation domain (IAD), critical in mediating protein-protein interactions *via* a linker domain (LKD) (Fig. 1) [6]. The DBD domain resembles a winged helix-turn-helix motif with a 3-helix bundle (α1, α2, α3), a 4-stranded antiparallel beta-sheet (β1–β4) and two large loops (between β2 and α2 and α2 and α3) (Fig. 1b). The third helix slots into the major groove of the 5′-GAAA-3′ subsequence and is the major determinant of sequence-specific binding through contacts made by arginine residues on the hydrophilic face with the phosphate backbone (Fig.1b). Three of the five invariant tryptophan residues contact DNA [12,6].

Unlike other IRF protein, IRF4 binds DNA with low affinity and requires further protein-protein interactions to bind DNA [6]. IRF4 is essential for the expression of both GC B cell-specific and PC-specific genes and the low affinity for DNA is thought to be central to this role. Depending on its protein levels, IRF4 binds DNA as a heterodimer or a homodimer to different motifs, each motif uniquely activating the expression of genes related to GC B cell or PC differentiation. At low protein levels, IRF4 binds as a heterodimer either the Ets-IRF composite elements EICEs (GGAANN(N)GAAA) with PU.1 (Fig. 2a, d) or the AP-1-IRF composite elements AICEs (GAAATGAGTCA or GAAANNNNTGAG TCA) with AP-1 family such as Batf (Fig. 2b, e) [13,14]. During the differentiation of B cell into PCs protein levels increase and IRF4 binds as a homodimer to the interferon sequence response elements ISREs (GAAANNGAAA) (Fig. 2c, f) [15].

The low DNA binding affinity of IRF4 has been attributed to the inhibitory activity of the last 30 residues of the IAD domain [16,13] (Fig. 1a). It has been postulated that this auto-inhibitory region (AR) prevents the DBD from binding to DNA, whilst DBD interactions with transcription factor partners would release AR inhibition [13]. This hypothesis however does not explain how release of the inhibition would occur when IRF4 binds to ISRE sequences as a homodimer. Recent structural studies have shown that the AR region is a flexible unstructured peptide that does not dock into the IAD helical bundle, as seen in IRF3 [6,17,18]. Furthermore, the diversity in sequence homology and length of the IRF4 AR region, suggest that alternative mechanisms could induce IRF4 dimerization on DNA. Small-angle X-ray scattering (SAXS) studies of full length IRF4 suggests that the linker region (LKD) connecting the DBD and IAD domains most likely adopts a folded conformation able to interact with the domains located at either end of the molecule and that it may therefore play a role in the regulation of IRF4 activity [6] (Fig. 1b).

3. IRF4 role in transcriptional circuitry of GC B cells and plasma cells

IRF4 is the master regulator of two mutually antagonistic programmes of B and PC cells gene expression [15]. B cells play a fundamental role in the humoral immune response. During antigen-dependent activation, B cells can rearrange the constant region of the IgH region yielding antibodies with different effector functions by a process called class-switch recombination (CSR). Moreover, after antigen-dependent activation, mature B cells undergo somatic hypermutation (SHM), a process that alters the variable regions of the immunoglobulin in order to select B cells producing high affinity antibodies. SHM leads to the affinity maturation of B cells in germinal centres (GCs) that are transient structures within secondary lymphoid organs where B cells are selected based on their ability to produce high-affinity antibodies [19]. GCs are characterized by two compartments: the dark zone (DZ) where B cells proliferate extensively undergoing SHM and the light zone (LZ) in which B cells are selected on the basis of their affinity for the antigen. The GCs ultimately produce memory B cells and high-affinity, long-lived PCs characterized by high level of antibody secretion [20]. Molecular alterations occurring during early and late phases of B cell development can lead to the generation of lymphoid tumours.

According to the ‘‘kinetic model’’ proposed by Ochiai et al. [15]. IRF4 regulates CSR, SHM, the generation of GC B cells and PC differentiation in a temporal and dose-dependent manner [8,7,15]. Specifically, IRF4 levels appear to define cell fate decisions by coordinating binding partner- and DNA-binding activity.

In the early stages of the GC reaction IRF4 is present at low levels and its binding to AICE and EICE motifs up-regulates activation-induced cytidine deaminase (AID) expression. AID (encoded by the *AICDA* gene), an enzyme that creates mutations in DNA by deamination of cytosine base, is absolutely necessary for CSR and SHM [21]. IRF4 also activates B-cell lymphoma 6 protein (BCL6), a transcriptional repressor mainly required for GC formation and antibody affinity maturation (Fig. 3a) [15,22]. On the other hand, elevated levels of IRF4 during PC differentiation favour binding of IRF4 to the ISREs of direct target genes such as *PRDM1*, which encodes protein PRDM1 (also known as BLIMP1) a key component of the PC differentiation transcription programme [23,24]. The shift to ISREs binding therefore mediates activation of *PRDM1* and repression of *BCL6*, bringing the GC programme to an end and promoting the differentiation into PCs (Fig. 3b, c).

IRF4 is absolutely required for GC formation. Studies looking at the effect in mice of B cell specific knockdown of IRF4, show a failure in GC formation caused by insufficient induction of BCL6 *and AID* [15,8,7]. BCL6, which is highly expressed in GC B cells, facilitates their rapid