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The glucorcorticoid receptor is increased in *Atm*−/− thymocytes and in *Atm*−/− thymic lymphoma cells, and its nuclear translocation counteracts *c-myc* expression

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ABSTRACT

We have previously demonstrated that spontaneous DNA synthesis in immature thymocytes of *Atm*−/− mice is elevated, and that treatment with the glucocorticoid dexamethasone (Dex) attenuates this increased DNA synthesis and prevents the development of thymic lymphomas. Deregulation of *c-myc* may drive the uncontrolled proliferation of *Atm*−/− thymocytes, since upregulation of *c-myc* parallels the elevated DNA synthesis in the cells. In this study, we show that the glucocorticoid receptor (GR) is expressed at high levels in *Atm*−/− thymocytes and in *Atm*−/− thymic lymphoma cells, although serum glucocorticoid (GC) levels in *Atm*−/− mice are similar to those in *Atm*+/+ mice. In cultured *Atm*−/− thymic lymphoma cells treated with Dex, GR nuclear translocation occurs, resulting in suppression of DNA synthesis and *c-myc* expression at both the mRNA and protein levels. Interestingly, the GR antagonist RU486 also causes GR nuclear translocation, but does not affect DNA synthesis and *c-myc* expression in *Atm*−/− thymic lymphoma cells. As expected, RU486 reverses the suppressive effects of Dex on DNA synthesis and *c-myc* expression. Administration of Dex to *Atm*−/− mice decreases the elevated c-Myc protein levels in their thymocytes. These findings suggest that GC/GR signaling plays an important role in regulating *c-myc* expression in *Atm*−/− thymocytes and thymic lymphoma cells.

1. Introduction

Ataxia-telangiectasia (A-T) is an autosomal recessive disease caused by mutations in the human *Atm* (A-T mutated) gene [1]. Mutations in the *Atm* gene result in multisystem disorders, two of which are thymic atrophy and thymic lymphoma [2–4]. Thymocytes from *Atm*−/− mice display a reduction in mature CD 4 or CD8 single-positive (SP) T lymphocytes and an increase of immature double-positive (DP) thymocytes relative to control mice [2]. T-dependent immune responses are impaired in *Atm*−/− mice, but T-independent immune responses are intact, and B cells in young *Atm*−/− mice are functionally normal. By 4–5 months of age, all *Atm*−/− mice die of thymic lymphomas [2,4]. Although it is known that thymic lymphoma cells in *Atm* deficient mice have multiple chromosome aberrations [5], the molecular mechanism for *Atm*−/− thymic lymphomagenesis is still poorly understood.

We have shown that spontaneous DNA synthesis is dramatically elevated in developing thymocytes of *Atm*−/− mice [6]. This result suggests that one role of ATM is to suppress DNA synthesis in immature thymocytes, allowing terminal cell differentiation, and to prevent thymic lymphomagenesis.

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The *c-myc* gene encodes a transcription factor which was first identified as a cellular homologue of a transforming gene encoded by the avian myelecytomatosis virus, MC29 [7,8]. Increased c-Myc expression is a common event in retrovirally induced leukemias and lymphomas in many species, including humans [9,10]. In *c-myc* transgenic mice, lymphoid and myeloid tumors occur, and subsequent inactivation of *c-myc* in the same transgenic mice results in regression of the established tumors [11].

c-Myc plays a critical role in the regulation of cell proliferation, cell differentiation, and apoptosis [12]. c-Myc elevation stimulates cyclin D/cdk4/6 and cyclin E/cdk3 activity, and suppresses the cdk inhibitors p21 and p27, thereby promoting cell cycling [13]. *c-myc*−/− mouse embryos die at E10, showing that c-Myc is essential for early embryonic development [14]. Thymi in *c-myc*−/− chimeras are small and double negative (DN) thymocytes fail to develop efficiently into the DP subset, although the cells can progress through DN3 stage and complete TCR-β chain rearrangement [15]. In normal mice, proliferating DN thymocytes express c-Myc at high levels, DP thymocytes at lower levels, and SP thymocytes at intermediate levels [15]. These findings suggest that c-Myc plays a critical role in normal thymocyte development.

Glucocorticoids (GC) have many functions, and play a prominent role in thymocyte development [16–18]. Although the adrenal gland is the major source of GC, ectopic production of GC by thymic epithelial cells has been reported [19], suggesting that locally produced GC in the thymus may play a special role in thymocyte differentiation. The GC receptor (GR) contains two transactivation domains, a ligand-binding domain and a zinc-finger DNA-binding domain [17,18]. In the absence of hormone, the GR is sequestered in the cytosol, where it is bound to a 90-kDa heat shock protein [20] or to 14-3-3σ [21]. Upon ligand binding, the GR rapidly translocates to the nucleus, where it binds to specific DNA sequences called glucocorticoid responsive elements, or GREs, and activates or represses transcription of the corresponding genes [18]. It has been reported that GC inhibits NF-κB activity [22], resulting in downregulation of NF-κB target c-myc gene expression [23]. However, overexpression of *c-myc* in transgenic mice rescues Dex-induced DP thymocyte death [23,24]. It seems likely that NF-κB/*c-myc* signaling and the GC/GR system may functionally counteract one another in thymocyte development.

2. Experimental

2.1. Mice

The *Atm*−/− mice used in this study were created by Barlow et al. [2]. Heterozygous *Atm*+/− mice of this line were purchased from the Jackson Laboratory (Bar Harbor, ME), mated, and kept in the Animal Center at The University of Texas M.D. Anderson Cancer Center (UTMDACC), Science Park-Research Division. Genotyping was carried out by polymerase chain reaction, as described by Liao et al. [25]. The mice were bred in the institutional accredited specific pathogen-free animal facility under standard conditions with a 14:10 light/dark cycle and were given standard diet (Teklad 8640, Indianapolis, IN) and acidified RO water *ad libitum*. All studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of UTMDACC. The Assurance number is A-3343-01.

2.2. Treatment of the mice

Eight-week old *Atm*+/+ and *Atm*−/− mice were treated with 5 mg/kg Dex daily, via i.p. for 2 weeks. Control untreated mice received the same volume of normal saline. Thymocytes were isolated from Dex-treated and untreated *Atm*+/+ and *Atm*−/− mice at the end of the treatment period, and proteins extracted for Western blot analysis.

2.3. Cells and cell lines

Thymocytes were isolated from *Atm*+/+ and *Atm*−/− mice using routine methods [6]. *Atm*−/− thymic lymphoma cell lines were established from 28 individual tumors in *Atm*−/− mice, at 3–4 months of age, as described previously [26]. The cells were cultured in RPMI-1640 medium (Life Technologies Inc.) supplemented with 10% FBS (Biological Industries) and 5 ng/ml of interleukin-2 (IL-2) (Sigma). In some cases, *Atm*−/− thymic lymphoma cells were further subcloned by limiting dilution, to obtain more purified lines with DP (CD4+ CD8+ double positive) or DN (CD4− CD8− double negative) phenotype. The *Atm*−/− cell lines were designated as ATL-1, ATL-2, ATL-3, ect., up to ATL-28. The phenotypes of the ATL-1 and ATL-27 lines used in this study are DP and DN, respectively. The ASL-1 thymic leukemia cell line [26] was used as a GC-resistant control.

2.4. Treatment of the cells

To assess the activation state of the glucocorticoid receptor (GR) in ATL cells, and to measure the effects of Dex on c-Myc expression, ATL cells were cultured with or without Dex, or with or without the GR antagonist RU486, for different time periods. Whole cell proteins, or separate cytoplasmic and nuclear fractions, were then prepared from these cells for Western blot analysis.

2.5. Flow cytometry analysis

Single-cell suspensions of *Atm*−/− thymic lymphoma cells were prepared in HBSS buffer and stained with PE-conjugated anti-CD4 and FITC-conjugated anti-CD8 antibodies (PharMingen, San Diego, CA). Flow cytometric analysis was performed with a Coulter EPICS Elite flow cytometer (Backman Coulter) to measure ratios of DN, DP and SP cells.

2.6. [^{3}H] thymidine incorporation into DNA

Single-cell suspensions of *Atm*−/− thymic lymphoma cells (4×10^4 cells/well) were cultured in 96-well plastic tissue culture dishes at 37 °C for 16 h, with or without Dex or RU486. For the last 4 h of culture, 0.5 μCi of ^{3}H-TdR (Amersham Pharmacia Biotech, Piscataway, NJ) was added into each well. The cells were harvested and [^{3}H] thymidine incorporation into DNA measured in a scintillation counter (Packard, Meriden, CT). Results were expressed as mean counts per minute (cpm) ± S.D. in triplicate cultures.