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The role of the pro-apoptotic protein Siva in the pathogenesis of Familial Mediterranean fever: A structural and functional analysis

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ABSTRACT

Familial Mediterranean fever (FMF) is an autosomal, recessive disease, attributed to mutations in *MEFV* gene encoding pyrin, which is characterized by recurrent, acute and self-limiting attacks of fever as well as an increased neutrophil and monocyte apoptosis. Most disease-associated mutations in *MEFV* gene reside on the C-terminal PRYSPRY (B30.2) domain of pyrin, an area found to interact with the pro-apoptotic protein Siva. Because apoptotic events may be contributing to endogenous inflammation we hypothesized that mutations in pyrin may affect Siva-mediated apoptosis. The confirmation of this hypothesis would be of a great biological significance since it would be demonstrated a connection between apoptosis and inflammation. We used homology modeling to construct a 3-D model of Siva protein and the constructed model of Siva defined structural elements with potential of binding other proteins to induce apoptosis. Given that Siva protein binds pyrin as shown by transfection and immunoprecipitation experiments, apoptosis was assessed by FACS and Western blotting. No differences in rates of apoptosis in myeloid cells (THP-1) upon transfection with either wt pyrin or mutant forms of pyrin were found. Patients with FMF did not display any mutations in the Siva-1 (full length) gene. Siva-1 was not linked to pyrin in the major predicted FMF gene network constructed using a literature-curated gene signature for FMF. These results suggest that Siva-mediated unprovoked apoptosis is not likely to be involved in the pathogenesis of FMF.

1. Introduction

Familial Mediterranean fever (FMF, MIM 249100) is an autosomal recessive disorder [1] characterized by acute, self-limited attacks of fever and serosal inflammation [2], affecting mainly individuals of the Mediterranean basin. FMF is the prototype of a group of single-gene inherited diseases referred to as hereditary recurrent fevers. The gene causing FMF, named Mediterranean fever gene (*MEFV*), encodes a 781 amino acid protein named pyrin or marenostrin [3,4]. Pyrin is expressed in cytokine-activated monocytes, serosal fibroblasts, polymorphonuclear cells and granulocytes [5,6]. Human pyrin protein contains a ~90 aa N-terminal pyrin (PYD) domain, followed by the bZIP transcription factor basic domain and B-box zinc finger coiled coil domains and the ~200-residue C-terminal PRYSPRY (B30.2) domain, a prevalent protein–protein interaction motif [7].

Unraveling of the genetic background and the pathogenetic mechanisms governing FMF is ongoing. FMF-associated mutations are likely to cause an increase in IL-1β production. Because most FMF-associated mutations reside in the PRYSPRY domain of pyrin, the current pathogenetic mechanism postulates a direct interaction of C-terminal B30.2 (or PRYSPRY) domain of pyrin with the catalytic domains of caspase-1; this may modulate the IL-1β production in FMF by promoting the activation of caspase-1 and the subsequent IL-1β secretion [7]. We have previously constructed a refined 3-D model showing the interaction of the PRYSPRY domain with caspase-1 [8]. In this model, the "flexible loops" of caspase-1 appear to have no access to positions located distally to the binding cavity of PRYSPRY domain, which have been previously associated with mild disease [9]. Together, modeling data suggest that alternative pathogenic pathways leading to FMF needed to be explored.

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A protein called Siva was recently isolated and confirmed to be a pyrin-PRYSPRY domain interacting protein [10]; this is in accordance with our previously reported putative interaction between the pro-apoptotic protein Siva protein and the PRYSPRY domain [9]. These data prompted us to further explore the contribution of Siva to the pathogenesis of FMF by analyzing in detail potential interactions between these two proteins. Siva and pyrin are co-expressed in human neutrophils, monocytes, and synovial cells [11]. Siva gene gives rise to the full-length predominant form Siva-1 and a minor alternate form, Siva-2, lacking exon 2, which is the central region of the protein and, therefore, the pro-apoptotic properties of Siva-1 [12,13]. Siva interacts with the cytoplasmic tail of several TNF receptor family members (CD27, GITR, OX40 and 4-1BB) [13,14], which are known to regulate cell proliferation and death. Siva also interacts with and inhibits the function of some of the anti-apopototic BCL-2 family members [15].

To further elucidate whether interactions between Siva and pyrin may contribute to the pathogenesis of FMF, we sought to: (a) define the topology of Siva-1 with respect to its interaction with pyrin and its ligand(s); (b) explore the potential increase of Siva-induced apoptosis in the presence of the mutant forms of pyrin; and (c) search for mutations in the pyrin-binding domain of Siva in FMF patients.

2. Materials and methods

2.1. Patients

Ten FMF patients displaying a severe FMF phenotype, from unrelated families living in Crete were included in this study (for more details see [8]). Among them, two were homozygotes for the M694V mutation, three were heterozygotes for the same mutation and in 5 of them no mutations were found. The study was performed in the Laboratory of Internal Medicine of the University of Crete after obtaining the approval of the institutional committee (University Hospital of Heraklion) [8]. The DNA data were analyzed anonymously.

2.2. Plasmid constructs and recombinant DNA techniques

The pCMV-Tag3a plasmid vector (Invitrogen) was used for cloning both wild-type and various mutant forms of pyrin, thus generating vectors encoding for the c-myc-pyrin fusion protein full-length Siva-1 as well as Siva-2 were subcloned into the pEGFP-C1 vector (Clontech, Mountain View, CA), encoding for the GFP-Siva protein. All these constructs were provided by Dr. Deborah Gumucio (University of Michigan, Ann Arbor, MI).

Whole blood was collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes and genomic DNA was isolated by using the commercial kit PUREGENE (Gentra SYSTEMS, MN). The genomic DNA sequence of human Siva-1 gene (GenBank Accession No. NM_006427) was used to design gene-specific primers for PCR amplification of the genomic fragment corresponding to the Siva-1 N-terminal amphipathic helix. The upstream 5′-ATG AGG AAC AGG CAA TGG AC-3′ and the downstream 5′-ATG CCC AAG CGG AGC TGC CCC TTC-3′ primers were used for the PCR amplification. The amplification was carried out using a Taq polymerase provided by QIAGEN. The resulting PCR products were sequenced double-stranded according to the di-deoxy-chain termination method following the manufacturer's protocol (Sequenase, USB), using custom gene-specific primers. Agarose gel electrophoresis and other recombinant DNA methods were performed essentially as described in Sambrook et al. [16].

2.3. Reagents

The antibodies used in this study are commercially available: living colors A.v. Monoclonal Antibody (JL-8) [anti-green fluorescent protein (GFP) mouse antibody] was from Clontech and anti-c-myc rabbit antibody was from Sigma. Anti-pyrin goat antibody, anti-β actin mouse antibody, anti-Bcl-xl mouse antibody and all secondary antibodies (anti-rabbit, anti-mouse and anti-goat), were obtained from Santa Cruz Biotechnology. Chemical reagents were purchased from Sigma.

2.4. Cell culture conditions and co-transfection via electroporation

THP-1 (human monocytic leukemia) cell lines were from ATCC. These cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin (5000 IU/ml)/streptomycin (5000 μg/ml) (all from Gibco) and 0.5 mM β-mercaptoethanol (THP-1 cells only), in a humidified atmosphere of 5% CO_2, at 37 °C. THP-1 cells were transiently co-transfected with different c-myc-pyrin, Siva-1 and Siva-2 expression vectors using the Amaxa nucleofection technology according to the manufacturer's protocol (Amaxa, Cologne, Germany). Amaxa-manufactured green fluorescent protein (GFP) plasmid encoding was used as a positive control (0.5 μg DNA) to confirm transfection efficiency under fluorescence microscopy. Furthermore, transfection efficiency was estimated by Western blotting.

2.5. Detection of apoptosis in THP-1 cells by FACS analysis

THP-1 cells were transiently co-transfected with c-myc-pyrin (wt or mutants) and GFP-Siva-1 expression vectors and apoptosis was measured 24 h later by FACS-Annexin-PE staining using the Annexin-V kit (Pharmingen) according to the manufacturer's protocol. Briefly, 10^6 cells were washed 2× with cold PBS, resuspended in 1 ml binding buffer and 100 μl of the cell suspension was incubated with 5 μl PI (stains necrotic cells) or 2 μl Annexin-PE (stains apoptotic cells) for 15 min in the dark. At the end of the incubation time, 400 μl binding buffer were added and samples were analyzed using the Beckman-Coulter EPICS Elite flow cytometer (Coulter). The results were analyzed with the WinMDI 2.8 software. The results from FACS analysis were also confirmed with Western blotting using an anti-Bcl-xl (an anti-apoptotic protein) antibody.

2.6. Construction of Siva-1 domains' three-dimensional (3-D) model

Distant sequence homology studies and secondary structure prediction have lead to the identification of separate structural and functional domains. The Protein Data Bank [17] was searched to find homologous structural domains such as in 1TFK for the Zinc finger, as in 1HEY and 2Q60 for the B-Box domain and as in 1TF0 for the amphipathic α-helix. 3-D model structures were created using modeling software, by using homologous 3-D defined protein domains as templates [18]. The QUANTA-CHARMm program [19,20] was used to check the derived models for folding and packing errors in order to arrive to a refined combined model with optimal atom contacts.

2.7. Gene network analysis

Using a literature-curated gene signature for FMF [21,22], gene networks were constructed and identified important hubs using Ingenuity Gene Network Analysis. Pathways of highly interconnected genes were identified by statistical likelihood using the following equation:

$$\text{Score} = -\log_{10}\left(1 - \sum_{i=0}^{f-1} \frac{C(G,i)C(N-G,s-i)}{C(N,s)}\right)$$

where N is the number of genes in the network of which G are central nodes genes, for a pathway of s genes of which f are central node genes. $C(n,k)$ is the binomial coefficient. We considered statistically significant networks those with a p value $< 10^{-10}$.

3. Results

3.1. Analyzing the structural–functional domains of Siva-1 protein

More information on the putative involvement of the Siva-1 protein and its interaction with pyrin in the pathogenesis of Familial Mediterranean Fever could be derived by defining the structural domains of the Siva protein and their role in protein–protein interactions (i.e., pyrin, GITR, ligands). Three domains of Siva-1 have been defined: an N-terminal domain containing an amphipathic helix, partly not present in Siva-2, a putative B-box like PHD tripartite domain (not present in Siva-2), and a zinc finger-like domain (Fig. 1).

3.2. Interaction of Siva-1 with the PRYSPRY domain of pyrin and its effect on apoptosis of monocytic THP-1 cells

While Siva is a pro-apoptotic protein that interacts with the cytoplasmic tail of several TNF receptor family members, GITR is the only ligand of Siva expressed in cells also expressing pyrin (i.e., monocytes, neutrophils and macrophages [23,24]. Based on this information, we first sought to explore the hypothesis that an apoptotic pathway involving pyrin/Siva interactions could represent an alternative pathway leading to the development of FMF. Thus, we assumed that the inability of some mutant forms of pyrin to bind efficiently Siva-1 may result in an increased Siva-1/GITR binding, thus leading to the further induction of the GITR-GITRL apoptotic pathway (Fig. 2).

We had examined previously by co-immunoprecipitation the ability of Siva-1 or -2 to bind pyrin as well as various mutant forms of this protein upon transfection into HEK293 cells [25] and confirmed the recent findings of Balci-Peynircioglu et al. [10]. In an attempt to examine whether Siva-1, when co-transfected with mutant forms of pyrin, could alter the apoptosis levels in THP-1 human monocytic cells, THP-1 cells were transfected with FMF-associated mutations, located on the rim of the binding cavity of PRYSPRY domain (aa positions #680, 694, 761), at the bottom of the binding cavity (aa #688) or on the back external surface of the binding cavity (aa #726, 744). Using Western blot, we confirmed the successful co-transfection of Siva-1 and pyrin or pyrin's mutants in THP-1 cells (data not shown). Co-transfection of Siva-1 with mutant forms of pyrin does not cause an increase in apoptosis activation, although mutant forms of pyrin seem to slightly inhibit Siva-1 mediated apoptosis as assessed by FACS analysis (Fig. 3). Although the nucleofection process by itself caused high apoptosis in THP-1 cells, as demonstrated in the same Figure, the data obtained strongly suggest that there is no further increase in the percentage apoptosis when Siva-1 was co-transfected with pyrin mutants compared to wt pyrin. These results were also confirmed by Western blot analysis using an antibody against Bcl-xl, an anti-apoptotic transmembrane protein (data not shown). We used only Siva-1 for transfection experiments since it is known that Siva-1 induces apoptosis more effectively than Siva-2 [26].

3.3. Mutations of Siva-1 detected in FMF patients

The finding that no major difference in apoptosis of THP-1 cells is observed when Siva-1 is co-transfected with wt or mutants of pyrin prompted us to explore the possibility that some patients carry any mutations in the N-terminal amphipathic helix of Siva-1, a region known to interact with the PRYSPRY domain of pyrin. We performed PCR amplification of this region of Siva-1 in selected FMF patients from our Cretan cohort [8]. According to our sequencing data, the amplified fragment of Siva-1 from patients with clinical FMF contained no mutations. The possibility of an aberrant binding of the amphipathic helix of Siva-1 to pyrin due to the presence of a mutation remains to be elucidated using a larger cohort of FMF patients.

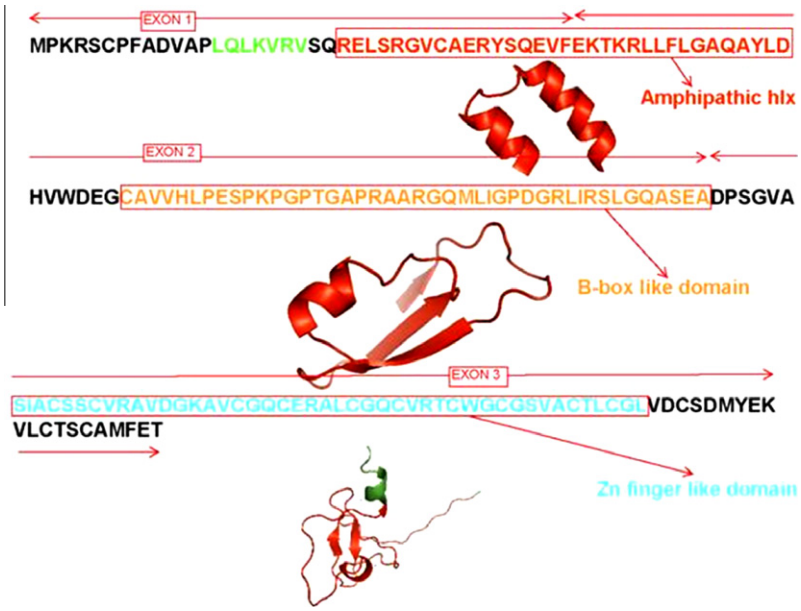

Fig. 1. Three-dimensional (3-D) components of Siva-1 protein. The domains are represented in red for the amphipathic helix domain partly missing in Siva-2, in green for a β-strand into this domain, in orange for the B-box like domain not present in Siva-2 and in cyan for the Zn finger-like domain. Exons are indicated by red arrows. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

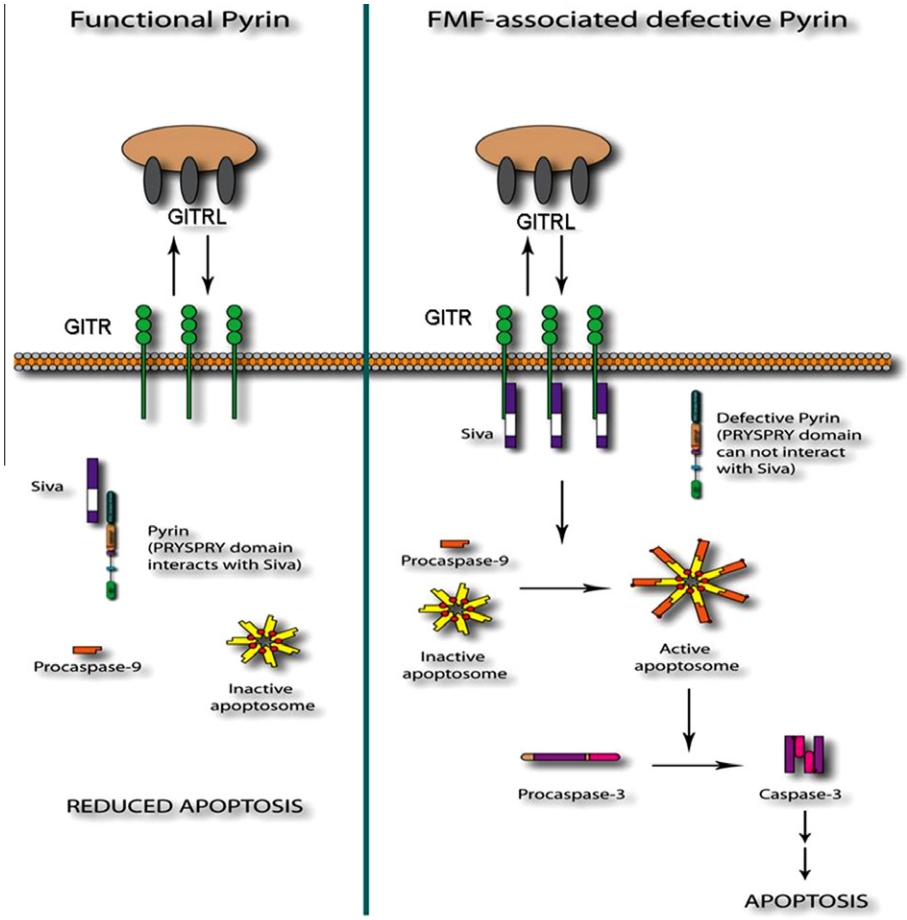

Fig. 2. Schematic representation of the pyrin/Siva-1/GITR proteins interactions, possibly leading to FMF. Siva-1 has an important role in the apoptotic pathway induced by the GITR protein. Binding of Siva-1 onto the PRYSPRY domain of pyrin may inhibit apoptosis (left side) but the various mutant forms of pyrin may not be able to bind Siva-1, thus contributing to the activation of the Siva-1/GITR mediated apoptosis.

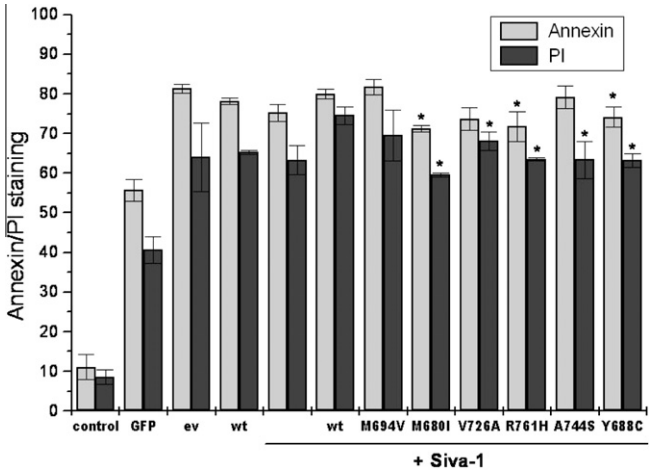

Fig. 3. Assessment of apoptosis in THP-1 cells. THP-1 cells were co-transfected with the indicated combinations of GFP-Siva-1 and c-myc-Pyrin (wt and mutants) via Nucleofection. PmaxGFP plasmid was used as a positive control of nucleofection under UV microscopy. Twenty-four hours after co-transfection of THP-1 cells with GFP-Siva-1- and c-myc-pyrin (wt or mutants), apoptosis was assessed by Annexin-PE staining and FACS analysis. PI staining was used to estimate necrotic cells. Results are mean ± SEM of at least three independent experiments and * denotes statistical significance ($p < 0.05$) compared to Siva-1 + wt pyrin.

3.4. Gene Network Analysis for Familial Mediterranean fever

To explore the putative role of Siva-1 protein in FMF pathogenesis, we used gene networks analysis. Based on a list of genes from the literature [21,22], we sought to correlate pyrin with other known pathways and identify potential new actions of pyrin. Central nodes in each network represent proteins that are key regulators of pyrin networks and could serve as potential drug targets. This analysis revealed two gene networks, however only one gene network was statistically significant ($p = 10^{-31}$). This network, which did not involve Siva-1, had central nodes the following genes: *NF-KB, IFNA, P38 MAPK, PI3K, CASP1, BCL2, MEFV, PYCARD* and *NLRP3* (Fig. 4). In contrast, the second gene involving Siva-1 but not displaying *MEFV* as a central node, did not reach statistical significance ($p = 10^{-4}$). These data suggest that to-date there is no evidence demonstrating a link of Siva-1 function to FMF.

4. Discussion

Pyrin plays a major role in the regulation of IL-1β secretion and the pathogenesis of FMF. However, some discrepancies remain that require further clarification. Identifying novel genes involved either in the regulation of *MEFV* gene or in potential interactions with pyrin protein may further delineate to pathogenic mechanisms in FMF. Since Siva protein interacts with the PRYSPRY (B30.2) domain of pyrin it was reasonable to assume that this

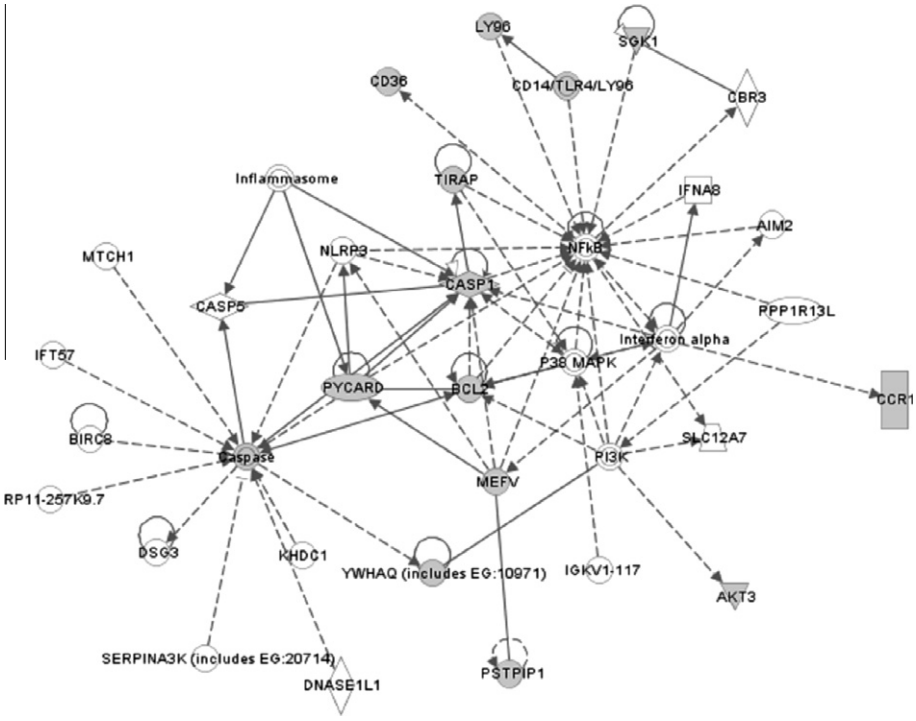

Fig. 4. Gene network analysis for Familial Mediterranean fever. Grey colors present the already known genes and in white the genes that the analysis predicted. Central nodes in each network are proteins that are central regulators of pyrin network and potentially can be drug targets.

interaction might contribute to the pathogenesis of FMF, causing or contributing to the inflammatory and/or apoptotic processes.

In our study apoptosis was assessed by FACS analysis and calculation of Bcl-xl levels. The data obtained by using various mutants of pyrin and Siva-1 for co-transfecting HEK293 [10,25] and THP-1 cells suggest that the positions of the PRYSPRY domain, found mutated in FMF patients, are not required for binding to Siva-1. Relative levels of apoptosis were monitored in separate experiments involving THP-1 cells, involving the same series of *MEFV* mutations appearing in FMF patients; the levels of apoptosis are comparable to those assessed after using the wt pyrin. It has been reported that oxidative stress by using H_2O_2 has a significant dampening effect of pyrin on Siva-1, thus suggesting that pyrin modulates Siva-induced apoptosis [10]. However, stimulation of the apoptotic cascade by using various triggers was beyond the scope of the present study since we were interested in mechanisms relevant to the FMF and not in *in vitro* treatments, which are not clearly involved in the pathogenesis of the disease.

Defining precisely the regions of the proteins involved in the pathogenesis of any disease is essential for the elucidation of the potential complexities or abnormalities in the conformation and interactions of these molecules. To clarify whether GITR (or another member of the TNF receptor family members) and pyrin antagonize for the same binding site(s) of Siva-1 we performed a domain analysis of the Siva-1 based on homology modeling. Three distinct domains of Siva-1 were defined, an N-terminal domain containing amphipathic helices with potential to interact with the PRYSPRY domain of pyrin, a B-box like domain and a zinc finger-like C-terminal domain that we assume to bind the cytoplasmic tail domain of GITR protein. A better understanding of the interaction of any of these domains with pyrin and apoptosis-mediating proteins may confer to the detection of distinct amino acid positions that play a crucial functional role. These findings may be of clinical importance, as they could facilitate the efforts to develop new therapeutic strategies by designing new inhibiting and/or mimicking molecules.

Despite recent major advances in unraveling of the pathophysiology of FMF, in some cases of the disease a further refinement of the existing pathways and mechanisms is required. Our ultimate goal was to explore the possibility of the involvement of a different pathogenetic mechanism in the development of FMF, apart from the already well-known IL-1β-mediated inflammatory procedure. However, we were not able to demonstrate an increase of Siva-induced apoptosis in the presence of the mutant forms of pyrin, thus suggesting that Siva is unlikely to contribute to the pathogenesis of FMF through the postulated molecular mechanism (Fig. 1).

The high number of heterozygote patients with typical clinical symptoms of FMF, possessing a single demonstrable *MEFV* mutation (something unexpected for a typical recessively inherited disease) has led to various hypotheses such as a digenic mode of inheritance, the existence of unidentified rare or non-exonic *MEFV* mutations, the function of genetic modifiers and the effect of environmental and/or epigenetic factors [27]. By using genes listed in the literature two gene networks were constructed; Siva-1 is not a central node in the network but is located in a distal position, suggesting that modulation of Siva-1 expression is not crucial for FMF pathogenesis. By using high throughput technologies involving either miRNAs or genome wide association studies (GWAS) aiming to define crucial SNPs (and the corresponding pathways), novel key molecules may be identified that may be related to *MEFV*-modifying and/or to pyrin-regulation genes.

5. Conclusion

Although the pyrin-interacting Siva protein was a putative mediator of the FMF development, the results suggest that Siva-mediated unprovoked apoptosis is not likely to be involved in the pathogenesis of FMF. The next step will be to use high throughput technology approaches to detect new molecules and subsequently new mechanisms that may refine further the molecular basis of the disease.

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References

[1] E. Sohar, J. Gafni, M. Pras, H. Heller, Familial Mediterranean fever. A survey of 470 cases and review of the literature, Am. J. Med. 43 (1967) 227–253.
[2] J. Samuels, S. Ozen, Familial Mediterranean fever and the other autoinflammatory syndromes: evaluation of the patient with recurrent fever, Curr. Opin. Rheumatol. 18 (2006) 108–117.
[3] The International FMF Consortium, Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever, Cell 90 (1997) 797–807.
[4] The French FMF Consortium, A candidate gene for familial Mediterranean fever, Nat. Genet. 17 (1997) 25–31.
[5] M. Centola, G. Wood, D.M. Frucht, J. Galon, M. Aringer, C. Farrell, D.W. Kingma, M.E. Horwitz, E. Mansfield, S.M. Holland, et al., The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators, Blood 95 (2000) 3223–3231.
[6] A. Diaz, C. Hu, D.L. Kastner, P. Schaner, A.M. Reginato, N. Richards, D.L. Gumucio, Lipopolysaccharide induced expression of multiple alternatively spliced MEFV transcripts in human synovial fibroblasts: a prominent splice isoform lacks the C-terminal domain that is highly mutated in familial Mediterranean fever, Arthritis Rheum. 50 (2004) 3679–3689.
[7] J.J. Chae, G. Wood, S.L. Masters, K. Richard, G. Park, B.J. Smith, D.L. Kastner, The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production, Proc. Natl. Acad. Sci. USA 103 (2006) 9982–9987.
[8] E. Fragouli, E. Eliopoulos, E. Petraki, P. Sidiropoulos, I. Aksentijevich, E. Galanakis, H. Kritikos, A. Repa, G. Fragiadakis, D.T. Boumpas, G.N. Goulielmos, Familial Mediterranean Fever in Crete: a genetic and structural biological approach in a population of 'intermediate risk', Clin. Genet. 73 (2008) 152–159.
[9] G.N. Goulielmos, E. Fragouli, I. Aksentijevich, P. Sidiropoulos, D.T. Boumpas, E. Eliopoulos, Mutational analysis of the PRYSPRY domain of pyrin and implications for familial Mediterranean Fever (FMF), Biochem. Biophys. Res. Commun. 345 (2006) 1326–1332.
[10] B. Balci-Peynircioglu, A.L. Waite, C. Hu, N. Richards, A. Staubach-Grosse, E. Yilmaz, D.L. Gumucio, Pyrin, product of the MEFV locus, interacts with the proapoptotic protein, Siva, J. Cell. Physiol. 216 (2008) 595–602.
[11] S. Papin, S. Cuenin, L. Agostini, F. Martinon, S. Werner, H.D. Beer, C. Grütter, M. Grütter, J. Tschopp, The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1 processing, Cell Death Differ. 14 (2007) 1457–1466.
[12] K.V. Prasad, Z. Ao, Y. Yoon, M.X. Wu, M. Rizk, S. Jacquot, S.F. Schlossman, CD27, a member of the tumor necrosis factor receptor family, induces apoptosis and binds to Siva, a proapoptotic protein, Proc. Natl. Acad. Sci. USA 9412 (1997) 6346–6351.
[13] B. Py, C. Slomianny, P. Auberger, P.X. Petit, S. Benichou, Siva-1, Siva-1 and an Alternative splice form lacking the Death Domain, Siva-2, similarly induce apoptosis in T lymphocytes via a caspase-dependent mitochondrial pathway, J. Immunol. 172 (2004) 4008–4017.
[14] S. Spinicelli, G. Nocentini, S. Ronchetti, L.T. Krausz, R. Bianchini, C. Riccardi, GITR interacts with the pro-apoptotic protein Siva and induces apoptosis, Cell Death Differ. 9 (2002) 1382–1384.
[15] F. Chu, A. Borthakur, X. Sun, J. Barkinge, R. Gudi, S. Hawkins, K.V. Prasad, The Siva-1 putative amphipathic helical region (SAH) is sufficient to bind to BCL-XL and sensitize cells to UV radiation induced apoptosis, Apoptosis 9 (2004) 83–95.
[16] J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Press, Cold Spring Harbour, New York, USA, 1989.
[17] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank, Nucleic Acids Res. 28 (2000) 235–242.
[18] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[19] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, CHARMM: a program for macromolecular energy minimization and dynamics calculations, J. Comput. Chem. 4 (1983) 165–175.
[20] Quanta, A molecular Graphics Program Licensed to Molecular Simulations, San Diego, CA, USA, 1998.
[21] G.M. Wood, J. Balow Jr., H.W. Sun, N. Shoham, D. Kastner, RNA interference of MEFV reveals potential genes regulated by pyrin, Arthritis Rheum. 56 (Suppl.) (2007) S629.
[22] G. Wood, J.J. Balow, H. Sun, N. Shoham, D. Kastner, RNA interference of MEFV in THP.1 cells reveals a role for endogenous pyrin in Toll-like receptor signaling (TLR) that is mediated by the transcription factor IRF2, Clin. Exp. Rheumatol. 26 (2008) 178.
[23] J. Shimizu, S. Yamazaki, T. Takahashi, Y.S. Sakaguchi, Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance, Nat. Immunol. 3 (2002) 135–142.
[24] L.T. Krausz, R. Bianchini, S. Ronchetti, K. Fettucciari, G. Nocentini, C. Riccardi, GITR-GITRL system, a novel player in shock and inflammation, Sci. World J. 7 (2007) 533–566.
[25] D. Vassou, E. Eliopoulos, E. Petraki, P. Sidiropoulos, E. Thymiakou, D. Gumucio, D. Kardassis, D.T. Boumpas, G.N. Goulielmos, Implication of the pro-apoptotic protein Siva in the pathogenesis of Familial Mediterranean Fever (FMF), Ann. Rheum. Dis. 68 (Suppl. 3) (2009) 179.
[26] H.H. Shin, S.J. Kim, S.Y. Kang, D.S. Lee, H.S. Choiet, Soluble glucocorticoid-induced tumor necrosis factor receptor stimulates osteoclastogenesis by down-regulation of osteoprotegerin in bone marrow stromal cells, Bone 39 (2006) 716–723.
[27] M.G. Booty, J.J. Chae, S.L. Masters, E.F. Remmers, B. Barham, J.M. Le, K.S. Barron, S.M. Holland, D.L. Kastner, I. Aksentijevich, Familial Mediterranean fever with a single MEFV mutation: where is the second hit?, Arthritis Rheum 60 (2009) 1851–1861.