FEBS Letters 586 (2012) 1795–1802

journal homepage: www.FEBSLetters.org

The NRF2-related interactome and regulome contain multifunctional proteins and fine-tuned autoregulatory loops

Diána Papp [a,b,1], Katalin Lenti [c,1], Dezső Módos [a,b,c], Dávid Fazekas [a], Zoltán Dúl [a], Dénes Türei [a,b], László Földvári-Nagy [a], Ruth Nussinov [d,e], Péter Csermely [b], Tamás Korcsmáros [a,b,*]

[a] Department of Genetics, Eötvös Loránd University, Budapest, Hungary
[b] Department of Medical Chemistry, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[c] Department of Morphology and Physiology, Faculty of Health Sciences, Semmelweis University, Budapest, Hungary
[d] Center for Cancer Research Nanobiology Program, Science Applications International Corporation (SAIC)-Frederick, Frederick National Laboratory, National Cancer Institute, Frederick, MD, USA
[e] Sackler Institute of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

ARTICLE INFO

Article history:
Received 13 February 2012
Revised 28 April 2012
Accepted 14 May 2012
Available online 26 May 2012

Edited by Paul Bertone

Keywords:
NRF2
Protein–protein interaction
Interaction prediction
Regulatory loops
Signaling network
Double-edged sword

ABSTRACT

NRF2 is a well-known, master transcription factor (TF) of oxidative and xenobiotic stress responses. Recent studies uncovered an even wider regulatory role of NRF2 influencing carcinogenesis, inflammation and neurodegeneration. Prompted by these advances here we present a systems-level resource for NRF2 interactome and regulome that includes 289 protein–protein, 7469 TF–DNA and 85 miRNA interactions. As systems-level examples of NRF2-related signaling we identified regulatory loops of NRF2 interacting proteins (e.g., JNK1 and CBP) and a fine-tuned regulatory system, where 35 TFs regulated by NRF2 influence 63 miRNAs that down-regulate NRF2. The presented network and the uncovered regulatory loops may facilitate the development of efficient, NRF2-based therapeutic agents.

1. Introduction

NRF2 (NF-E2-related factor 2, NFE2L2) is a master transcription factor involved in oxidative and xenobiotic stress responses [1]. NRF2 features a Cap "n" collar (CNC) basic leucine zipper (bZIP) structure that enables NRF2 to form heterodimer with the ZIP domain of small MAF proteins [2]. NRF2 has six conserved domains, designated as Neh1–6 (Nrf2-ECH homology) domains [3]. The complex domain structure allows NRF2 to bind to DNA and to multiple proteins including other transcription factors, the helicase- and chromodomain containing co-activator CHD6, the CREB-binding protein (CBP), and KEAP1, the major negative regulator of NRF2 [4–6]. NRF2 also contains several lysine and serine residues that serve as regulatory target sites for ligases and kinases, respectively [3].

Under normal conditions, NRF2 is inhibited by KEAP1. When cells are exposed to oxidative stress, electrophiles, or chemopreventive agents, NRF2 escapes KEAP1-mediated repression, enters the nucleus, forms a heterodimer with its obligatory partner MAF, and activates antioxidant responsive element (ARE)-dependent gene expression to maintain cellular redox homeostasis [3]. The cytoprotective role of NRF2 involves a cross-talk with other cellular processes. As an example of this the p53-regulated p21 directly activates NRF2 and promotes cell survival [7]. A key apoptosis and autophagy regulating signaling adaptor, p62 also induces NRF2 by inhibiting the basal KEAP1 repression [7]. Signaling pathways possibly involved in the regulation of NRF2 include MAPKs (mitogen-activated protein kinases), PI3K (phosphatidylinositol 3-kinase), protein kinase C (PKC) and CK2 (casein kinase 2) related pathways [8]. Activation of NRF2 is a well-known adaptive response to environmental and endogenous stresses. Recent studies uncovered that NRF2 influences a wide range of physiologic and pathologic processes such as inflammation, carcinogenesis, chronic obstructive pulmonary disease, obesity, and neurodegeneration [9].

Despite the wide range of NRF2-driven processes, the interactors and regulators of NRF2 have not been studied at the systems-level. While there are more than 2300 articles for the keyword 'NRF2' in PubMed (as of January 2012), major protein–protein interaction resources (BioGRID, MINT, STRING, HPRD) contain only a few (10–20) interactors for NRF2. Besides one study on the global

* Corresponding author.
E-mail address: korcsmaros@netbiol.elte.hu (T. Korcsmáros).
[1] Equal contributions.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.05.016

mapping of binding sites for NRF2 through ChIP-Seq profiling [10] and a specific network modeling approach on Nrf2 regulation in mouse lung [11], there is no large-scale collection of NRF2-regulated target genes. Prompted by the lack of systems-level NRF2-related information we collected literature information on NRF2 interacting proteins and regulated genes, as well as predicted novel NRF2 interactors and regulators. Moreover, we compiled a list of potential NRF2 regulating transcription factors and miRNAs (i.e., NRF2 regulome). Finally, we imported datasets from external sources to achieve higher coverage and benchmark the collected database. This database allowed us to examine network motifs and regulatory loops [12] in the NRF2 interactome and regulome (Fig. 1a). NRF2 is a promising anticancer agent, and its unregulated activation enhances tumor cell protection against chemotherapy [13]. Thus, the detailed understanding of the complexity of NRF2 interactome and regulome may fine-tune NRF2-related anticancer approaches.

2. Materials and methods

2.1. Building the NRF2 interactome and regulome

For the manual curation of the NRF2 interactome, we applied a curation protocol which is similar to the one that we have previously developed for the manual curation of 8 major signaling pathways in 3 organisms [14]. To identify additional interactors for NRF2, we predicted protein–protein interactions based on possible domain–domain and domain–motifs interactions. The protocol which was applied during the manual curation and the details of the predictions can be found in *Supplementary* Material 1. We downloaded additional NRF2 interactions already deposited in BioGRID, MINT, HPRD and IntAct by using the webservice of PathwayCommons [15]. As NRF2 is important in the inflammation process [9], we also downloaded its interactors and target genes from InnateDB [16]. To predict target genes regulated by NRF2, we applied the transcription factor binding site (TFBS) information of NRF2 from JASPAR [17]. We also included all experimentally verified NRF2 target genes from a large-scale ChIP-Seq profiling study [10]. In order to predict transcription factors (TFs) that directly regulate *NRF2* transcription, we applied a reverse approach, and queried the regulatory region of the *NRF2* gene sequence against all TFBS information found in JASPAR [17]. Additionally, we acquired the list of miRNAs predicted to regulate NRF2 mRNA from miRBase [18]. Finally, we mined the resources TransMir and PutMir [19,20] to identify those TFs that regulate the transcription of miRNAs known to regulate NRF2 (acquired at the previous step from miRBase).

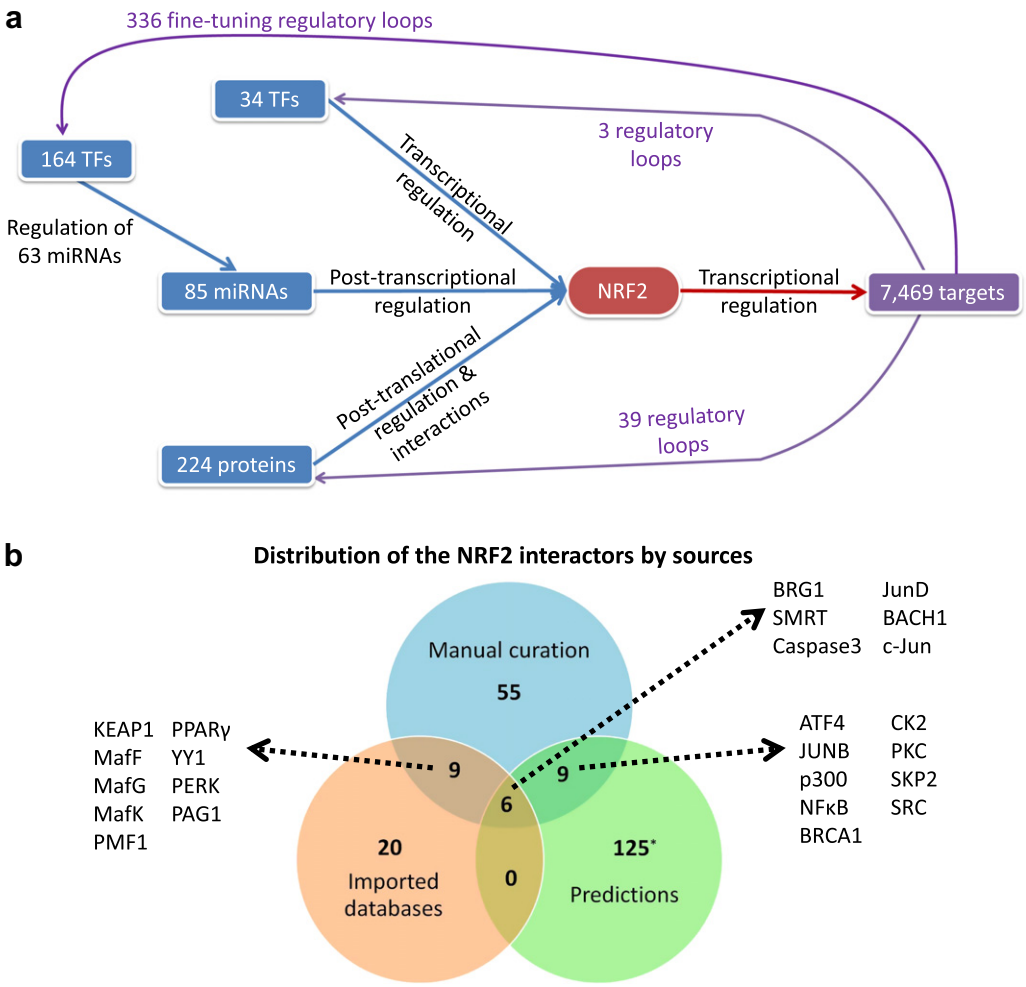

Fig. 1. Components and interaction types of the NRF2 interactome and regulome. (a) Structure of the database with the numbers of the components and the regulatory loops. Regulators of NRF2 are shown with blue color. Purple arrows highlight the number of those target genes that regulate NRF2 (termed as regulatory loops). (b) Venn-diagram of the source types used to build the NRF2 interactome. Numbers represent the number of NRF2 interactions/interactors from the given source type. The "imported databases" represent the following sources: BioGRID, MINT, IntACT, HPRD and Innate DB. "Predictions" represent both domain–motif and domain–domain interactions. The asterisk notes that from the 125 predicted interactions we found 13 already described in the literature (but not found during the manual curation step).

3. Results and discussion

3.1. The NRF2 interactome

We created a high-definition (HD) NRF2 interactome by manually curating the literature. This HD interactome contains 108 proteins, 131 directed and 15 undirected interactions (Fig. 2). Though during the curation we also focused on KEAP1, the major regulator of NRF2 [3], we found only 17 KEAP1-related interactions (11.6%). We observed that the majority of the identified interactions (57%, 84) were NRF2-interactions. 42% (55) of the 131 directed interactions were inhibitory, while 58% (76) were activating interactions. We found the molecular mechanisms, for example dimerization and phosphorylation, for 76 direct interactions from the total 146 interactions. For the remaining 70 interactions only the corresponding activating or inhibiting effect were provided in the literature. We searched for possible domain–motif and domain–domain interactions between interacting protein pairs to predict underlying molecular mechanisms. Based on this and earlier literature searches, we defined 12 interactions as 'predicted as direct' and the remaining 58 as 'indirect'. Note that some of these indirect interactions can be direct but currently there is no literature- or structure-based evidence to support this. Thus, the created high-definition interactome contains NRF2-interactors with known functional role in the NRF2 network. The HD NRF2 interactome contains molecular details for each interaction (e.g., dimerization, phosphorylation) and the whole dataset is available in *Supplementary* Material 2.

To extend the coverage of the HD interactome, we predicted and imported additional interactors for NRF2. We identified 22 directed and 121 undirected interactions based on domain–motif and domain–domain interaction predictions, respectively. These interactions were found probable and highly-confident by the ELM structure filter [21] or by the PRINCESS PPI-evaluation tool [22], respectively. For the PRINCESS evaluation we defined a very stringent confidence value at 1000 to filter the total 1427 predicted PPIs to 121 highly-confident interactions. Furthermore, we checked the literature and found 13 domain–motif and 14 domain–domain interactions validating the predictions. Thus, the remaining 9 and 107 interactions can be considered as 116 newly predicted NRF2-interactions. Another extension for the NRF2 interactome was the integration of 28 interactions from PPI databases and 20 interactions from InnateDB. We note that for the predicted and imported interactors less functional details are available compared to the interactors in the HD interactome. But these additional interactions could point out less known interactors, whose experimental analysis may help the understanding of the pleiotropic function of NRF2. The integrated NRF2 interactome can be found in *Supplementary* Material 3.

To benchmark the links of the integrated NRF2 interactome we compared all interactions from the different sources. We found 6 interactions present in three sources and 18 in two sources. Two

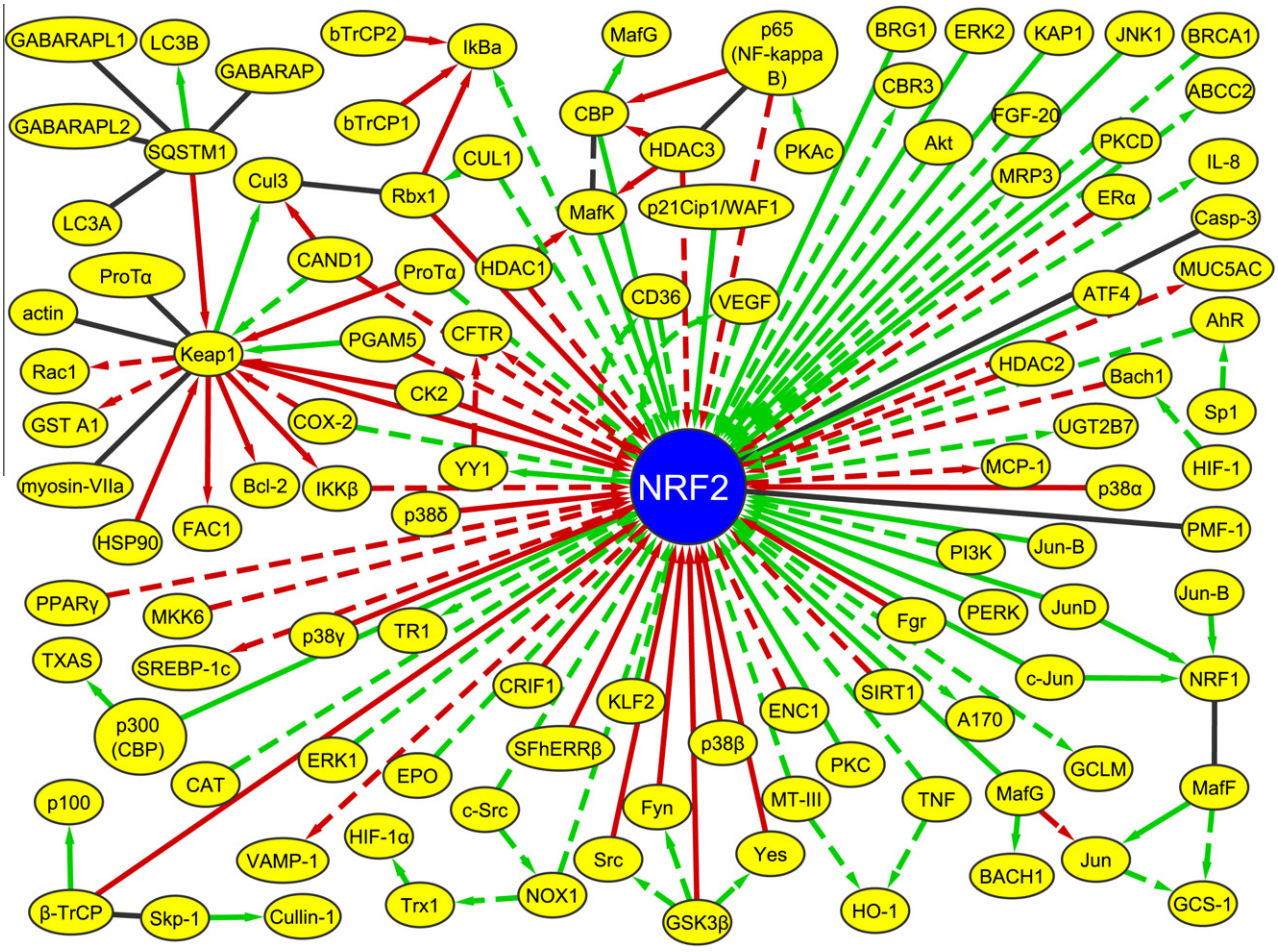

Fig. 2. Manually curated network of NRF2. The high-density NRF2 interactome with interactions among NRF2 interactors. Activation links are shown in green, inhibitory links are shown in red color. Undirected interactions have gray color. Direct interactions are presented with solid lines, indirect interactions with dashed lines. See Fig. 4 for an enlarged subnetwork and Supplementary material 8 for a searchable network image.

hundred interactions were found only in one source. Altogether we found 224 identical interactions (i.e., interactors) for NRF2 from the different sources (Fig. 1b). The low overlap between the manually curated and imported interactions shows that the currently existing databases lack most of the interactions that have already been described in the literature. The high number of high-confidence predictions without any overlap with the other sources indicates many possible novel interactors. In addition, the overlap between the predicted and the experimentally described interactors (found in manual curation and in PPI databases) shows that 10% of the predictions have already been validated.

3.2. Functional analysis of the NRF2 interactome

We analyzed the GO biological processes [23] of the NRF2 interactors both in the HD and the integrated NRF2 interactome, and found 8 major processes. As we found the same ranks for the two interactomes ($p = 1$; Wilcoxon ranksum test), we present the analysis on the HD interactome in detail (Fig. 3). In the HD interactome approximately 30–35 interactors of NRF2 were involved in the same 5 processes; that is, signaling, stress, response to chemical stimulus, metabolism and development. Except development these major functions are generally known for NRF2 [7]. These analyses showed that one-third of the NRF2 interactors were highly multifunctional. Furthermore, we found 27 proteins involved in the immune system with smaller overlap with the other processes, as well as 15 and 16 interactors involved in reproduction and wound healing, respectively. The dataset and enrichment statistics used for the functional analysis can be found in *Supplementary* Material 4.

In spite of the high number of NRF2 interactors having developmental functions (42 proteins), there is no exact description on the role of NRF2 in mammalian development. In 1996, Chan et al. [24] showed that NRF2 is not essential in developmental processes but later this have been challenged [25–27]. SKN-1, the *Caenorhabditis elegans* ortholog of NRF2, is known to be important in the mesoderm and endoderm formation [28]. SKN-1 was found to be involved in oocyte maturation [29], while NRF2 have recently been described to take part in spermatogenesis [30], suggesting the importance of NRF2 in reproduction; however, reproduction is not yet among the GO annotations of NRF2. Similarly, immune-related processes are also missing from the GO annotations of NRF2 though 32% of the NRF2 interactors have immune functions and NRF2 is known to be involved in innate immunity [16]. We and others recently proved the role of SKN-1 in the pathogen response of *C. elegans* suggesting a high-level functional similarity between NRF2 and SKN-1 [31,32].

3.3. The NRF2 regulome

NRF2 expression is regulated by transcription factors (TF) and miRNAs. Accordingly, we predicted 34 TFs that could regulate the expression of NRF2 based on TF–TFBS binding data from JASPAR [17]. We checked the literature and found 11 predicted TFs (MEF2A, ESR1, ESR2, NF-kappaB, PPARG, SP1, NFE2L2 and the TF-complexes MYC-MAX, EWSR1-FLI1) which are already known to regulate NRF2 showing that the prediction algorithm can indeed identify valuable regulatory connections. The remaining 24 predicted TFs, such as FOXA1, STAT1, PAX6 can be regarded as promising novel regulators for experimental validation. The miRBase resource [18] contained 85 identical miRNAs predicted to bind to NRF2 mRNA and down-regulate its translation. Thus, we found altogether 34 TFs and 85 miRNAs that could directly regulate the expression of NRF2 (for a complete list, see Supplementary Material 5). Till now, regulation of NRF2 was mostly considered as a post-translational mechanism via KEAP1 [3]. Here, we point out numerous TFs that may be involved in the regulation of the expression of NRF2 and these TFs could be important in the basal function of NRF2 [10] as well as in the long-time effect of NRF2 upon induction. Down-regulating NRF2 by miRNAs have already been described for a few miRNAs (e.g., miR-28, miR-144) and the malfunction of this regulation is linked to different pathological states [33,34]. The predicted NRF2 regulators need to be analyzed experimentally under normal and in diseased conditions to validate the prediction.

We also collected the target genes of NRF2. First, by manual curation we found 29 NRF2 target genes in the literature. We extended this list by including 6 target genes from the manually curated InnateDB and 1054 genes identified in a large-scale ChIP-Seq study [10]. Based on TF–TFBS binding data from JASPAR [17], we further predicted 6426 NRF2 target genes. We found some overlaps between the compiled 7515 target genes: while no genes present in all four sources, we found 1 target gene (COX6C) in three sources (manual curation, ChIP-Seq, JASPAR), and 44 target genes in two sources. Thus, altogether we found 7469 identical target genes for NRF2 from which 7424 target genes were found only by one source. The small overlap among the sources, especially between the large-scale ChIP-Seq study and the JASPAR prediction was surprising but can be explained by checking the details of these methods. The examined sequence regions were much broader in the prediction (see Supplementary Material 1) and the ChIP-Seq study contained only confident, functional and NRF2-specific target sequences [10]. Thus, the prediction may contain non-functional NRF2 binding sites but it may also list those binding sites that are bound by NRF2 in complex with other transcription factors

Functional overlap between NRF2 interactors	Development	Stress	Metabolism	Response to chemical stimulus	Signaling	Immune system	Reproduction	Wound healing
Development	**42**							
Stress	32	**42**						
Metabolism	37	36	**48**					
Response to chemical stimulus	29	29	30	**37**				
Signaling	31	32	36	29	**41**			
Immune system	22	25	24	22	25	**27**		
Reproduction	16	13	14	12	13	10	**16**	
Wound healing	13	15	12	10	12	9	4	**15**

Fig. 3. Functional overlap in the NRF2 interactome. The 8 major Gene Ontology Biological Processes are shown among the NRF2 interactors. The numbers in the main diagonal represent the total number of the NRF2 interactors involved in the given function, while the numbers in the matrix represent the overlap between the given function. The colors of the cells illustrate the level of the overlap between the functional groups. Two functions, "immune system" and "reproduction" is highlighted with red as they are not among the NRF2 Gene Ontology terms (see Supplementary material 4. for the whole dataset and functional analysis).

containing homeodomain or bZIP domains. In addition, the ChIP-Seq study only listed target genes whose expression was positively regulated by NRF2 [10], while the predicted set may contain negatively regulated genes. Detailed expression studies will be necessary to determine the effect of NRF2 on the expression of these possible target genes.

Combining the upstream and downstream components of the NRF2 regulatory network resulted in an integrated regulome, containing the regulators as well as the regulated genes of NRF2. The integrated regulome can be found in Supplementary Material 5.

3.4. Interaction and regulatory loops

Using the developed integrated resource for NRF2, we searched for network motifs, i.e., loops among the interacting proteins, target genes and NRF2 regulating TFs, and miRNAs. In the high-density interactome we found 7 bidirectional interactions (i.e., two-component feedforward and feedback-loops) between NRF2 and 7 of its interactors (BRCA1, KEAP1, NFE2, BACH1, ATF4, CUL3, NCOR1). In the same network we identified 23 three-component feedforward-loops among NRF2 and its 27 interactors (Fig. 4). In nearly all feedforward-loops NRF2 is located at the final, executive position, suggesting that there are several mechanisms that regulate NRF2 directly and also indirectly. When the signal (e.g., activation) is the same – in both direct and indirect mechanisms – then NRF2 will coherently and functionally 'correctly' act; on the other hand, when the signal is incoherent (e.g., a direct inhibition followed by an indirect activation) it could modulate and adapt its activity for the specific functional requirements [35]. For example, the loop containing CK2, KEAP1 and NRF2 is such an incoherent feedforward loop. Here, CK2 fulfils a key role in the control of NRF2 as it directly inhibits NRF2 only under specific conditions [36]. Interestingly, we found no three-component feedback loops in the interactome suggesting that this important regulatory mechanism is not characteristic to the NRF2-related protein network.

We combined the list of NRF2 interactors from the integrated interactome and the list of NRF2 target genes from the integrated regulome to identify two-component regulatory feedback-loops between them. We found 39 proteins that interact with NRF2 and their expression is regulated by NRF2 (Supplementary Material 6). For example, JNK1 can phosphorylate NRF2, which induces its translocation into the nucleus, where NRF2 transcriptionally induces the expression of stress-responsive genes [37] that include the JNK1 itself [38] (Fig. 5a). Similarly, p300/CBP, an important transcriptional co-activator, directly acetylates NRF2 which augments its promoter-specific DNA binding [39]. As an extension of our current knowledge on the CBP-NRF2 connection, we predicted that NRF2 can regulate the expression of CBP (Fig. 5b). In conclusion, nearly 20% (39 of 224) of the NRF2 interacting proteins have regulatory feedback connections with NRF2. This feedback mechanism could serve as amplification (in the case of a positive feedback) or signal down-regulation (in the case of a negative feedback). Based on available microarray data [10,40,41] and details on the NRF2 interactions, we found 10 positive and 3 negative feedback loops (listed in Supplementary Material 6). For the remaining 26 feedbacks, no data were available on the transcriptional effect of NRF2 on its target genes (activation or inhibition) or on the role of the protein interacting with NRF2. Further studies are needed to determine the effect of these interactions. Experimental analysis of the identified 13 feedback loops would help to understand their regulatory functions in the cellular network.

A similar search of the NRF2 regulating TFs and NRF2 target genes identified 3 TFs, the nuclear hormone receptors PPARγ and RORA, and the transcription factor, NFIL3 forming 3 regulatory

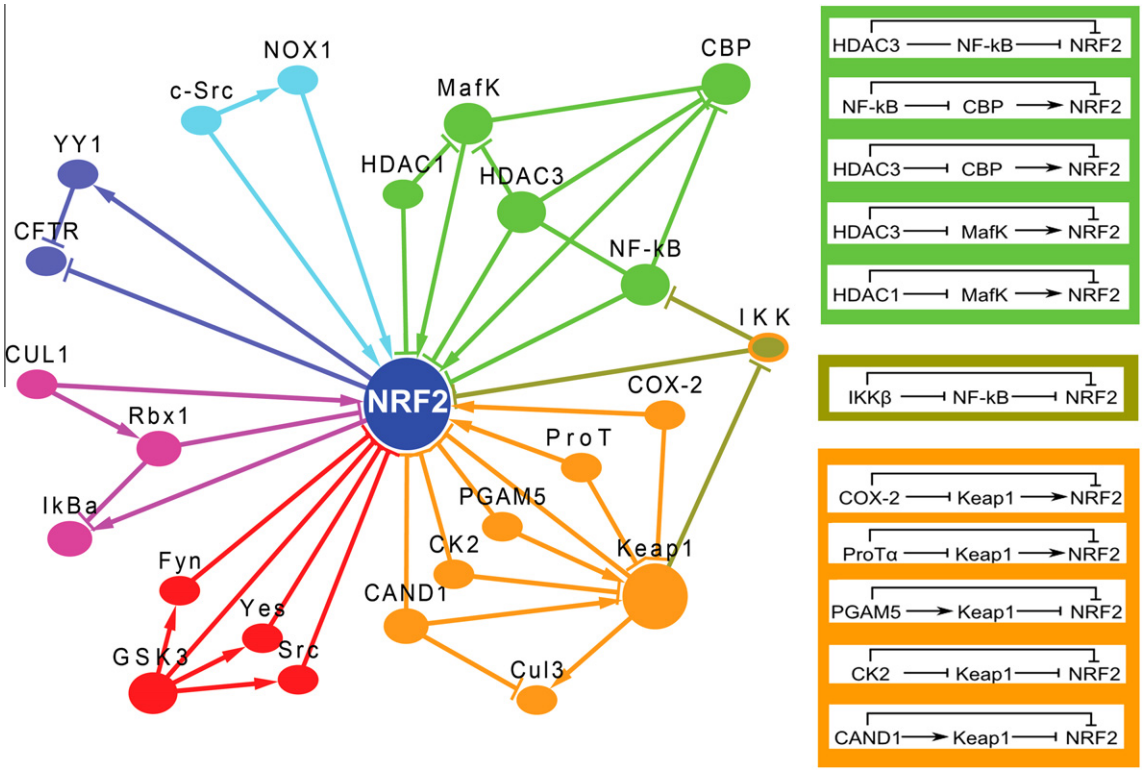

Fig. 4. Feedforward loops in the manually curated NRF2 interactome. The subnetwork of the HD NRF2 interactome containing only feedforward interaction loops. Activation links are shown with delta arrows, inhibitory links with T-arrows, while undirected interactions have no arrows. Direct interactions are presented with solid lines, indirect interaction with dashed lines. Note the 4 smaller loops marked with different colors on the left side of the image and the more complex intertwined motif-system with KEAP1 (orange) and NF-kB (green) on the right side, enlarged in colored boxes.

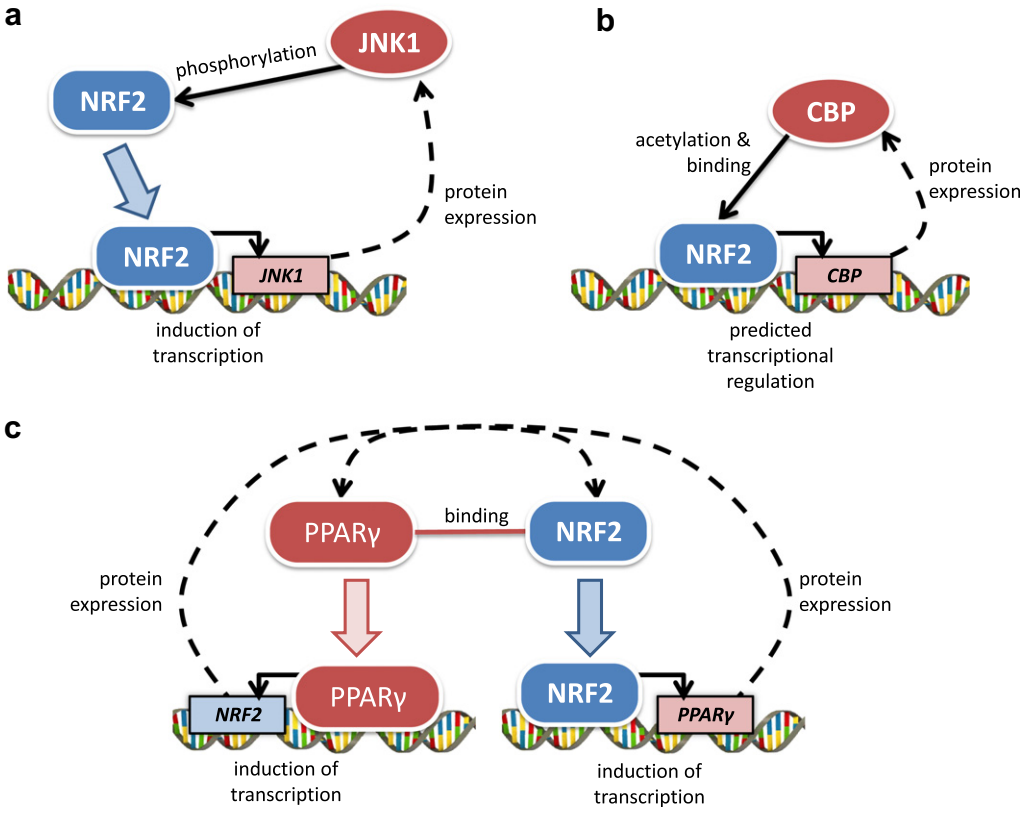

Fig. 5. NRF2 regulatory loops. (a) The regulatory loop of NRF2 and JNK1. (b) The regulatory loops of NRF2 and CBP, where the NRF2 regulation is predicted. (c) The mutual regulatory loop between NRF2 and PPARγ.

loops with NRF2 (Fig. 1a). NRF2 binds to the promoter of PPARγ and stimulates its transcription [42]. PPARγ and NRF2 have already been predicted to form a mutual feedback regulation [43]. We also note that PPARγ can directly interact with NRF2 [44] indicating a more complex regulatory mechanism between the two TFs (Fig. 5c). We found no literature evidence on any connections between NRF2 and NFIL3 or between NRF2 and RORA. Thus, these regulatory loops are lucrative targets for further experimental inquiries. The major function of NFIL3 is to transcriptionally regulate genes important in the immune response [45]. The mutual feedback loop between NRF2 and NFIL3 may point out an important cross-talk between the anti-oxidant and immune responses. RORA is a multi-functional protein that bridges inflammation and metabolism [46]. The mutual feedback loop between NRF2 and RORA could bi-directionally coordinate the changes of cellular processes during inflammation.

Finally, we searched for three-component regulatory loops among NRF2 regulating miRNAs, the TFs that regulate these miRNA and NRF2 target genes. We found 164 TFs that regulate NRF2 regulating miRNAs. The comparison with the NRF2 target genes showed 35 TFs, whose expression is regulated by NRF2 while they regulate altogether 63 miRNAs that down-regulate NRF2 (Fig. 1a). We found that the transcription of 18 TFs (from the 35) is activated by NRF2 with no data for the remaining 17 TFs. Altogether 74% (63 of 85) of the NRF2-regulating miRNAs could serve as feedback loops. Combining available data on the transcriptional effects of NRF2 and the miRNA regulating TFs, we found only 1 TF, TWIST1 that represses a possible anti-NRF2 miRNA (miR-200a), while the other TFs possibly activate the miRNAs. Therefore, most of the regulatory loops serve as a negative feedback for NRF2 (activating a TF that activates a down-regulating miRNA) but positive feedback loops can also exist, where the activated TF represses a down-regulating miRNA. The difference between the number of simple TF-loops (containing 3 TFs) and the more complex, miRNA-loops (containing 336 possible loops between 35 TFs and 63 miRNAs) point to a fine-tuned NRF2-regulatory system. We believe that the complexity of this regulatory system allows tissue and stress specific responses as well as the integration of the NRF2-related responses to other cellular processes. Similar systems have already been suggested for other master TF regulators, such as DAF-16/FOXO and the heat shock factor HSF-1 [47–49]. Dynamical expression studies on the key 63, NRF2 regulating miRNAs might uncover this mechanism. We listed all regulatory loops in Supplementary Material 7.

4. Conclusions

We developed a systems-level resource for NRF2, containing manually curated, predicted and imported physical as well as regulatory interactions. All data sources can be examined separately with their corresponding available evidence or confidence scores. These allow the users selection of data types and confidence levels. As our manual curation contained more than three times the number of interactions for NRF2 than the already existing databases, and 81% of the predicted interactions are novel, we believe that our compilation could provide an efficient resource for the biomedical research on NRF2. This resource could facilitate network pharmacological attempts [50], including a multi-target concept [51,52] and the recently proposed allo-network drug concepts [53] and may help to overcome challenges in targeting NRF2 which is a double-edged sword in many diseases; that is, the activation of NRF2 in healthy cells could delay or prevent the onset of some forms of human cancers [54], while its constitutive activation is responsible for acquired chemoresistance in tumor cells [13,55]. Thus, we believe that a more detailed, dynamical understanding

of the NRF2 interactome, regulome and fine-tuned regulatory loops will help to develop more potent NRF2 activator and inhibitor therapeutic agents.

Acknowledgements

We thank the anonymous reviewers for their advice and G. Szuromi for technical help. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number HHSN261200800001E, by the EU-ESF grant TAMOP 4.2.1./B-09/1/KMR-2010-0003 and TAMOP-4.2.2/B-10/1-2010-0013, the Hungarian Scientific Research Fund (OTKA K83314), the Hungarian Research and Technology Office (5LET-08-2-2009-0041) and by a residence at the Rockefeller Foundation Bellagio Center for PC. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.05.016.

References

[1] Moi, P., Chan, K., Asunis, I., Cao, A. and Kan, Y.W. (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc. Natl. Acad. Sci. U.S.A. 91, 9926–9930.
[2] Motohashi, H. and Yamamoto, M. (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol. Med. 10, 549–557.
[3] Zhang, D.D. (2006) Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab. Rev. 38, 769–789.
[4] Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D. and Yamamoto, M. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 13, 76–86.
[5] Nioi, P., Nguyen, T., Sherratt, P.J. and Pickett, C.B. (2005) The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. Mol. Cell Biol. 25, 10895–10906.
[6] Katoh, Y., Itoh, K., Yoshida, E., Miyagishi, M., Fukamizu, A. and Yamamoto, M. (2001) Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes Cells 6, 857–868.
[7] Taguchi, K., Motohashi, H. and Yamamoto, M. (2011) Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells 16, 123–140.
[8] Surh, Y.J., Kundu, J.K. and Na, H.K. (2008) Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med. 74, 1526–1539.
[9] Kensler, T.W., Wakabayashi, N. and Biswal, S. (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol. 47, 89–116.
[10] Malhotra, D., Portales-Casamar, E., Singh, A., Srivastava, S., Arenillas, D., Happel, C., Shyr, C., Wakabayashi, N., Kensler, T.W., Wasserman, W.W. and Biswal, S. (2010) Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Res. 38, 5718–5734.
[11] Taylor, R.C., Acquaah-Mensah, G., Singhal, M., Malhotra, D. and Biswal, S. (2008) Network inference algorithms elucidate Nrf2 regulation of mouse lung oxidative stress. PLoS Comput. Biol. 4, e1000166.
[12] Barabasi, A.L. and Oltvai, Z.N. (2004) Network biology: understanding the cell's functional organization. Nat. Rev. Genet. 5, 101–113.
[13] Palli, D., Vineis, P., Russo, A., Berrino, F., Krogh, V., Masala, G., Munnia, A., Panico, S., Taioli, E., Tumino, R., Garte, S. and Peluso, M. (2000) Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. Int. J. Cancer 87, 444–451.
[14] Korcsmaros, T., Farkas, I.J., Szalay, M.S., Rovo, P., Fazekas, D., Spiro, Z., Bode, C., Lenti, K., Vellai, T. and Csermely, P. (2010) Uniformly curated signaling pathways reveal tissue-specific cross-talks and support drug target discovery. Bioinformatics 26, 2042–2050.
[15] Cerami, E.G., Gross, B.E., Demir, E., Rodchenkov, I., Babur, O., Anwar, N., Schultz, N., Bader, G.D. and Sander, C. (2011) Pathway commons, a web resource for biological pathway data. Nucleic Acids Res. 39, D685–D690.
[16] Lynn, D.J., Chan, C., Naseer, M., Yau, M., Lo, R., Sribnaia, A., Ring, G., Que, J., Wee, K., Winsor, G.L., Laird, M.R., Breuer, K., Foroushani, A.K., Brinkman, F.S. and Hancock, R.E. (2010) Curating the innate immunity interactome. BMC Syst. Biol. 4, 117.
[17] Portales-Casamar, E., Thongjuea, S., Kwon, A.T., Arenillas, D., Zhao, X., Valen, E., Yusuf, D., Lenhard, B., Wasserman, W.W. and Sandelin, A. (2010) JASPAR 2010: the greatly expanded open-access database of transcription factor binding profiles. Nucleic Acids Res. 38, D105–D110.
[18] Kozomara, A. and Griffiths-Jones, S. (2011) MiRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 39, D152–D157.
[19] Wang, J., Lu, M., Qiu, C. and Cui, Q. (2010) TransmiR: a transcription factor-microRNA regulation database. Nucleic Acids Res. 38, D119–D122.
[20] Bandyopadhyay, S. and Bhattacharyya, M. (2010) PuTmiR: a database for extracting neighboring transcription factors of human microRNAs. BMC Bioinformatics 11, 190.
[21] Dinkel, H., Michael, S., Weatheritt, R.J., Davey, N.E., Van Roey, K., Altenberg, B., Toedt, G., Uyar, B., Seiler, M., Budd, A., Jodicke, L., Dammert, M.A., Schroeter, C., Hammer, M., Schmidt, T., Jehl, P., McGuigan, C., Dymecka, M., Chica, C., Luck, K., Via, A., Chatr-aryamontri, A., Haslam, N., Grebnev, G., Edwards, R.J., Steinmetz, M.O., Meiselbach, H., Diella, F. and Gibson, T.J. (2012) ELM – the database of eukaryotic linear motifs. Nucleic Acids Res. 40, D242–D251.
[22] Li, D., Liu, W., Liu, Z., Wang, J., Liu, Q., Zhu, Y. and He, F. (2008) PRINCESS, a protein interaction confidence evaluation system with multiple data sources. Mol. Cell Proteomics 7, 1043–1052.
[23] Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25, 25–29.
[24] Chan, K., Lu, R., Chang, J.C. and Kan, Y.W. (1996) NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proc. Natl. Acad. Sci. U.S.A. 93, 13943–13948.
[25] Chan, J.Y., Kwong, M., Lu, R., Chang, J., Wang, B., Yen, T.S. and Kan, Y.W. (1998) Targeted disruption of the ubiquitous CNC-bZIP transcription factor, Nrf-1, results in anemia and embryonic lethality in mice. EMBO J. 17, 1779–1787.
[26] Farmer, S.C., Sun, C.W., Winnier, G.E., Hogan, B.L. and Townes, T.M. (1997) The bZIP transcription factor LCR-F1 is essential for mesoderm formation in mouse development. Genes Dev. 11, 786–798.
[27] Lee, J.M., Chan, K., Kan, Y.W. and Johnson, J.A. (2004) Targeted disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia. Proc. Natl. Acad. Sci. U.S.A. 101, 9751–9756.
[28] Maduro, M.F., Kasmir, J.J., Zhu, J. and Rothman, J.H. (2005) The Wnt effector POP-1 and the PAL-1/Caudal homeoprotein collaborate with SKN-1 to activate *C. elegans* endoderm development. Dev. Biol. 285, 510–523.
[29] Lin, R. (2003) A gain-of-function mutation in oma-1, a *C. elegans* gene required for oocyte maturation, results in delayed degradation of maternal proteins and embryonic lethality. Dev. Biol. 258, 226–239.
[30] Nakamura, B.N., Lawson, G., Chan, J.Y., Banuelos, J., Cortes, M.M., Hoang, Y.D., Ortiz, L., Rau, B.A. and Luderer, U. (2010) Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. Free Radic. Biol. Med. 49, 1368–1379.
[31] Papp, D., Csermely, P. and Soti, C. (2012) A role for SKN-1/Nrf in pathogen resistance and immunosenescence in *Caenorhabditis elegans*. PLoS Pathog. 8, e100267.
[32] Hoeven, R., McCallum, K.C., Cruz, M.R. and Garsin, D.A. (2011) Ce-Duox1/BLI-3 generated reactive oxygen species trigger protective SKN-1 activity via p38 MAPK signaling during infection in *C. elegans*. PLoS Pathog. 7, e1002453.
[33] Yang, M., Yao, Y., Eades, G., Zhang, Y. and Zhou, Q. (2011) MiR-28 regulates Nrf2 expression through a Keap1-independent mechanism. Breast Cancer Res. Treat. 129, 983–991.
[34] Sangokoya, C., Telen, M.J. and Chi, J.T. (2010) MicroRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. Blood 116, 4338–4348.
[35] Bleris, L., Xie, Z., Glass, D., Adadey, A., Sontag, E. and Benenson, Y. (2011) Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. Mol. Syst. Biol. 7, 519.
[36] Pi, J., Bai, Y., Reece, J.M., Williams, J., Liu, D., Freeman, M.L., Fahl, W.E., Shugar, D., Liu, J., Qu, W., Collins, S. and Waalkes, M.P. (2007) Molecular mechanism of human Nrf2 activation and degradation: role of sequential phosphorylation by protein kinase CK2. Free Radic. Biol. Med. 42, 1797–1806.
[37] Xu, C., Yuan, X., Pan, Z., Shen, G., Kim, J.H., Yu, S., Khor, T.O., Li, W., Ma, J. and Kong, A.N. (2006) Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. Mol. Cancer Ther. 5, 1918–1926.
[38] Owuor, E.D. and Kong, A.N. (2002) Antioxidants and oxidants regulated signal transduction pathways. Biochem. Pharmacol. 64, 765–770.
[39] Sun, Z., Chin, Y.E. and Zhang, D.D. (2009) Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol. Cell Biol. 29, 2658–2672.

[40] Cho, H.Y., Reddy, S.P., Debiase, A., Yamamoto, M. and Kleeberger, S.R. (2005) Gene expression profiling of NRF2-mediated protection against oxidative injury. Free Radic. Biol. Med. 38, 325–343.
[41] Thimmulappa, R.K., Mai, K.H., Srisuma, S., Kensler, T.W., Yamamoto, M. and Biswal, S. (2002) Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res. 62, 5196–5203.
[42] Pi, J., Leung, L., Xue, P., Wang, W., Hou, Y., Liu, D., Yehuda-Shnaidman, E., Lee, C., Lau, J., Kurtz, T.W. and Chan, J.Y. (2010) Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity. J. Biol. Chem. 285, 9292–9300.
[43] Cho, H.Y., Gladwell, W., Wang, X., Chorley, B., Bell, D., Reddy, S.P. and Kleeberger, S.R. (2010) Nrf2-regulated PPAR{gamma} expression is critical to protection against acute lung injury in mice. Am. J. Respir. Crit. Care Med. 182, 170–182.
[44] Ikeda, Y., Sugawara, A., Taniyama, Y., Uruno, A., Igarashi, K., Arima, S., Ito, S. and Takeuchi, K. (2000) Suppression of rat thromboxane synthase gene transcription by peroxisome proliferator-activated receptor gamma in macrophages via an interaction with NRF2. J. Biol. Chem. 275, 33142–33150.
[45] Motomura, Y., Kitamura, H., Hijikata, A., Matsunaga, Y., Matsumoto, K., Inoue, H., Atarashi, K., Hori, S., Watarai, H., Zhu, J., Taniguchi, M. and Kubo, M. (2011) The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. Nat. Immunol. 12, 450–459.
[46] Jetten, A.M. (2009) Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nucl. Recept. Signal 7, e003.
[47] Libina, N., Berman, J.R. and Kenyon, C. (2003) Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell 115, 489–502.
[48] Pirkkala, L., Nykanen, P. and Sistonen, L. (2001) Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. 15, 1118–1131.
[49] Doshi, B.M., Perdrizet, G.A. and Hightower, L.E. (2008) Wound healing from a cellular stress response perspective. Cell Stress Chaperones 13, 393–399.
[50] Hopkins, A.L. (2008) Network pharmacology: the next paradigm in drug discovery. Nat. Chem. Biol. 4, 682–690.
[51] Csermely, P., Agoston, V. and Pongor, S. (2005) The efficiency of multi-target drugs: the network approach might help drug design. Trends Pharmacol. Sci. 26, 178–182.
[52] Korcsmaros, T., Szalay, M.S., Bode, C., Kovacs, I.A. and Csermely, P. (2007) How to design multi-target drugs: Target-search options in cellular networks. Expert Opin. Drug Disc. 2, 799–808.
[53] Nussinov, R., Tsai, C.J. and Csermely, P. (2011) Allo-network drugs: harnessing allostery in cellular networks. Trends Pharmacol. Sci. 32, 686–693.
[54] Martin-Montalvo, A., Villalba, J.M., Navas, P. and de Cabo, R. (2011) NRF2, cancer and calorie restriction. Oncogene 30, 505–520.
[55] Lau, A., Villeneuve, N.F., Sun, Z., Wong, P.K. and Zhang, D.D. (2008) Dual roles of Nrf2 in cancer. Pharmacol. Res. 58, 262–270.