CORE
Metadata, citation and similar papers at core.ac.uk
Provided by University of Dundee Online Publications

University of Dundee

Oncogene-Stimulated Congestion at the KEAP1 Stress Signaling Hub Allows Bypass of NRF2 and Induction of NRF2-Target Genes that Promote Tumor Survival

Hayes, John D.; Dinkova-Kostova, Albena T.

Published in:
Cancer Cell

DOI:
10.1016/j.ccell.2017.10.009

Publication date:
2017

Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):
Hayes, J. D., & Dinkova-Kostova, A. T. (2017). Oncogene-Stimulated Congestion at the KEAP1 Stress Signaling Hub Allows Bypass of NRF2 and Induction of NRF2-Target Genes that Promote Tumor Survival. Cancer Cell, 32(5), 539-541. https://doi.org/10.1016/j.ccell.2017.10.009

General rights
Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Oncogene-stimulated congestion at the KEAP1 stress signaling hub allows bypass of NRF2 and induction of NRF2-target genes that promote tumor survival

John D. Hayes and Albena T. Dinkova-Kostova

Jacqui Wood Cancer Centre

Division of Cancer Research

Ninewells Hospital and Medical School

University of Dundee

Dundee DD1 9SY

Scotland

UK

Correspondence: John D. Hayes, Jacqui Wood Cancer Centre, James Arrott Drive, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland, United Kingdom; e-mail, j.d.hayes@dundee.ac.uk; phone, +44 (0)1382 383182; Fax, +44 (0)1382 386419.

Funding: The authors are supported by grant MR/N009851/1 from the Medical Research Council of the UK and grant C20953/A18644 from Cancer Research UK.

© 2017. This manuscript version is made available under the CC-BY-NC-ND 4.0 license
http://creativecommons.org/licenses/by-nc-nd/4.0/

Abstract/summary

In this issue of Cancer Cell, Ge et al. show that overexpression of the oncoprotein iASPP in cancer cells provokes NRF2-mediated induction of cytoprotective genes because it logjams the ubiquitin ligase substrate adaptor function of KEAP1 by virtue of the fact it possesses a novel DLT-containing KEAP1-interaction motif.

Main text

The inhibitor of apoptosis stimulating protein of p53 (iASPP) is regarded as an oncoprotein because it antagonizes the ability of p53 to induce pro-apoptotic genes (see Lu et al. (2013) and papers cited therein). Since p53 regulates expression of antioxidant enzymes, such as catalase, GPX1 and SOD2, it might be anticipated that its inhibition upon overexpression of iASPP would increase the intracellular levels of reactive oxygen species (ROS). Contrary to such expectations, Ying Hu and colleagues discovered that ectopic overexpression of iASPP in cancer cell lines decreased levels of ROS and increased the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG), in a p53-independent manner, whereas knockdown of iASPP increased ROS levels and decreased the ratio of GSH to GSSG (Ge et al., 2017).

In an attempt to understand how iASPP decreased level of intracellular ROS and increased antioxidant capacity, Ge et al. considered that it might activate the cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factor NF-E2 p45-related factor 2 (NRF2, encoded by *NFE2L2*) because NRF2 is a master regulator of redox homeostasis. In response to oxidative stressors, NRF2 induces genes involved in the synthesis and maintenance of both glutathione- and thioredoxin-based antioxidant systems as well as the detoxification of drugs. Consistent with this proposal, these workers found that knockdown of iASPP in human bladder T24 carcinoma cells decreased expression of endogenous NRF2-target genes whilst overexpression of iASPP induced

NRF2-regulated genes. Moreover, the increase in NRF2-target gene expression affected by iASPP was accompanied by an increase in the abundance of NRF2 protein, but not NRF2 mRNA, and was therefore attributed to inhibition of degradation of the CNC-bZIP transcription factor (Ge et al., 2017).

To test whether iASPP might interfere with NRF2 protein turnover directed by Kelch-like ECH-associated protein 1 (KEAP1) and the cullin-3 (CUL3)-RING ubiquitin ligase CRL^{KEAP1}, Ge et al. demonstrated iASPP can physically interact with KEAP1 and in so doing blocks binding of NRF2 to KEAP1. These experiments revealed that a DLT motif in iASPP, between amino acids 239 and 241, is required for its interaction with the Kelch-repeat domain (also called DGR) of KEAP1. They then provided experimental evidence that the interaction of iASPP with KEAP1 prevented CRL^{KEAP1} from ubiquitylating NRF2, thereby stabilizing the CNC-bZIP transcription factor and enabling induction of its target genes (Ge et al., 2017).

It is well known that KEAP1 is a CUL3 substrate adaptor and that NRF2 binds to the two Kelch-repeat domains in the dimeric KEAP1 protein through two separate motifs in its N-terminal Neh2 domain, designated DLG and ETGE (**Figure 1A**). The ETGE motif binds to KEAP1 with a relatively high affinity (dissociation constant approx. 10 nM), whereas the DLG motif binds to KEAP1 with lower affinity (dissociation constant approx. 3200 nM) (Fukutomi et al., 2014). Importantly, both motifs are required for CRL^{KEAP1} to ubiquitylate NRF2 (McMahon et al., 2006). Besides NRF2, other proteins can bind KEAP1. Ben Major and colleagues identified a significant number of proteins with ETGE or related motifs that bind KEAP1 (Hast et al. 2013) (**Figure 1B**), a finding that suggests KEAP1 represents the hub of a network of pathways that links various agonists to NRF2-mediated induction of antioxidant and detoxication genes. In particular, NRF2 activity can be increased by some of these ETGE-containing proteins, when they are overexpressed or phosphorylated, because they prevent NRF2 from docking simultaneously through both its DLG and ETGE motifs onto dimeric KEAP1 (**Figure 1C**); it is envisaged that

the ETGE-containing proteins out-compete the low-affinity DLG motif of NRF2 for binding to KEAP1.

The best studied example of cross-talk through the KEAP1 signaling hub is provided by p62/sequestosome 1 (SQSTM1). During nutritional stimuli, the STGE motif of p62/SQSTM1 is phosphorylated by mechanistic target of rapamycin complex 1 (mTORC1), whereupon its affinity for KEAP1 is increased, resulting in formation of a p62/SQSTM1-KEAP1 complex that is removed by selective autophagy, thereby allowing induction of NRF2-target genes (Ichimura et al., 2013). Another example, which shares some parallels with iASPP, is that of the ETGE-containing protein dipeptidyl peptidase 3 (DPP3), which when overexpressed in squamous lung or breast cancer cells stimulates an increase in NRF2 activity; notably, the interaction between DPP3 and KEAP1 is bolstered by treatment with H_2O_2 (Hast et al., 2013; Lu et al., 2017). Further examples of ETGE-containing proteins that bind KEAP1 and possibly influence NRF2 activity include the breast cancer protein PALB2 (partner and localizer of BRCA2) and the DNA helicase minichromosome maintenance 3 (MCM3), but as both PALB2 and MCM3 are ubiquitylated by CRL^{KEAP1} (Orthwein et al.,2015; Mulvaney et al., 2016) their effects on NRF2-target gene expression may be short-lived.

A remarkable feature of the binding of iASPP to KEAP1 is that it involves a DLT motif. This was unexpected because all proteins identified to date that bind KEAP1 possess a 'high-affinity' ETGE motif, or closely related sequence, and none contain only a 'low-affinity' DLG motif (**Figure 1B**). It is therefore unclear how the DLT motif in iASPP can compete with NRF2 for binding to KEAP1. It appears likely that the DLT motif in iASPP forms a novel interaction structure and that flanking sequences increase the ability of iASPP to bind to KEAP1. This notion is supported by the fact that in cancer (particularly in non-small cell lung carcinoma), many somatic mutations in *NFE2L2* that result in NRF2 gain-of-function produce amino acid substitutions in the Neh2 domain between residues 23 and 43, suggesting that amino acids other

than the DLG tripeptide contribute to binding of this motif to KEAP1 (Fukutomi et al., 2014). Indeed, Fukutomi et al. (2014) pointed out that hydrophobic interactions are particularly important in the binding of DLG-containing peptides to the Kelch-repeat domain of KEAP1. On the basis of this argument, residues in iASPP, such as R244 may contribute to binding by the DLT motif to KEAP1, as will hydrophobic residues (**Figure 1B**). Provocatively, the DLT motif in iASPP lies between two PP dipeptide sequences (amino acids 232/233 and 246/247) that may impart a unique fold on this region of iASPP, but the significance of these two PP residues is unknown.

The fact that iASPP can bind KEAP1 raises interesting questions about its relationship with other proteins engaged in the KEAP1 signaling hub. Firstly, since p62/SQSTM1 is capable of sequestering KEAP1 for autophagy, can it similarly direct iASPP for autophagy when iASPP is bound to KEAP1? Secondly, since iASPP inhibits CRL^{KEAP1} ubiquitylation of NRF2, does it also inhibit CRL^{KEAP1} ubiquitylation of MCM3, PALB2 and PGAM5? Thirdly, what biological implications does the binding of iASPP to KEAP1 have for the association of other proteins that may not be ubiquitylated by CRL^{KEAP1}?

Having described the mechanism by which iASPP positively controls NRF2, Ge at el (2017) showed that knockdown of iASPP in xenograft models resulted in a reduction in NRF2 protein, and expression of its target genes, along with a decrease in tumor weight and size. Moreover, knockdown of iASPP in xenograft models increased the sensitivity of the tumors to the chemotherapeutic drug 5-fluorouracil.

In summary, the work of Ge et al (2017) suggests that iASPP co-opts NRF2 to support an oncogenic program that increases cell proliferation and drug resistance. In particular, iASPP delegates the task of augmenting antioxidant defenses and drug detoxification to NRF2. It is also possible that NRF2 aids iASPP-directed oncogenesis by reprograming metabolism. Further work is required to establish the extent to which iASPP contributes to NRF2 activity in normal and

cancer tissue, and the extent to which the overexpression of iASPP in tumors perturbs the KEAP1 stress signaling network.

Selected reading

Fukutomi, T., Takagi, K., Mizushima, T., Ohuchi, N., and Yamamoto, M. (2014) Mol. Cell. Biol. *34*, 832-846.

Ge, W., Zhao, K., Wang, X., Li, H., Yu, M., He, M., Xue, X., Zhu, Y., Zhang, C., Cheng, Y., Jiang, S., and Hu, Y. (2017) Cancer Cell in press

Hast, B.E., Goldfarb, D., Mulvaney, K.M., Hast, M.A., Siesser, P.F., Yan, F., Hayes, D.N., and Major, M.B. (2013) Cancer Res. *73*, 2199-2210.

Ichimura, Y., Waguri, S., Sou, Y.S., Kageyama, S., Hasegawa, J., Ishimura, R., Saito, T., Yang, Y., Kouno, T., Fukutomi, T., Hoshii, T., Hirao, A., Takagi, K., Mizushima, T., Motohashi, H., Lee, M.S., Yoshimori, T., Tanaka, K., Yamamoto, M., and Komatsu, M. (2013) Mol. Cell. *51*, 618-631.

Lu, K., Alcivar, A.L., Ma, J., Foo, T.K., Zywea, S., Mahdi, A., Huo, Y., Kensler, T.W., Gatza, M.L., and Xia, B. (2017) Cancer Res. *77*, 2881-2892.

Lu, M., Breyssens, H., Salter, V., Zhong, S., Hu, Y., Baer, C., Ratnayaka, I., Sullivan, A., Brown, N.R., Endicott, J., Knapp, S., Kessler, B.M., Middleton, M.R., Siebold, C., Jones, E.Y., Sviderskaya, E.V., Cebon, J., John, T., Caballero, O.L., Goding, C.R., and Lu, X. (2013) Cancer Cell. *23*, 618-633.

Ma, J., Cai, H., Wu, T., Sobhian, B., Huo, Y., Alcivar, A., Mehta, M., Cheung, K.L., Ganesan, S., Kong, A.N., Zhang, D.D., and Xia, B. (2012) Mol. Cell. Biol. *32*, 1506-1517.

McMahon, M., Thomas, N., Itoh, K., Yamamoto, M., and Hayes, J.D. (2006) J. Biol. Chem. *281*, 24756-24768.

Mulvaney, K.M., Matson, J.P., Siesser, P.F., Tamir, T.Y., Goldfarb, D., Jacobs, T.M., Cloer, E.W., Harrison, J.S., Vaziri, C., Cook, J.G., and Major, M.B. (2016) J. Biol. Chem. *291*, 23719-23733.

Orthwein, A., Noordermeer, S.M., Wilson, M.D., Landry, S., Enchev, R.I., Sherker, A., Munro, M., Pinder, J., Salsman, J., Dellaire, G., Xia, B., Peter, M., and Durocher, D. (2015) Nature. *528*, 422-426.

Figure 1. Identity of members of the KEAP1 stress signaling hub

(A) The cartoon illustrates the two-site tethering of NRF2, through its DLG and ETGE motifs, across two Kelch-repeat domains contained within the KEAP1 dimer. Both the DLG and ETGE motifs have to lock onto the KEAP1 dimer for NRF2 protein ubiquitylation, and subsequent proteasomal degradation, to proceed (McMahon et al., 2006).

(B) Amino acid sequences in human NRF2 that encompass the DLG and ETGE motifs (shown underlined at the top) have been aligned with sequences of proteins that interact with KEAP1. The alignment is based solely on the presence of DLG-related, ETGE, DTGE, ESGE and STGE sequences within proteins reported to bind KEAP1. The amino acids in NRF2 that are shown as white letters on a black background are those that have been reported to have been changed by somatic *NFE2L2* mutations in cancer and are therefore presumed to be necessary for its interaction with KEAP1 (Fukutomi et al., 2014). Amino acids in the other KEAP1-binding proteins that align with these residues in NRF2 are depicted in the same way. In all cases, data from human proteins are presented.

(C) Cartoon showing stimuli that are postulated to enhance the binding of iASPP, p62/SQSTM1, DPP3 and PALB2 for KEAP1. iASPP is overexpressed during oncogenesis (Ge et al., 2017). p62/SQSTM1 is phosphorylated at a STGE motif by mTORC1 in response to nutrients (Ichimura et al., 2013). DPP3 is overexpressed during oncogenesis and its interaction with KEAP1 is also increased by oxidative stress (Lu et al., 2017). PALB2 is involved in DNA double-strand break repair. Although forced overexpression of PALB2 can activate NRF2 (Ma et al., 2012), it is unclear whether this happens during stimulation of DNA damage because PALB2 is ubiquitylated by CRL^{KEAP1} (Orthwein et al., 2015); this uncertainty is indicated by a question mark. Through their KEAP1-binding activity, iASPP, p62/SQSTM1, DPP3 and [possibly] PALB2 can each stabilize *de novo* synthesized NRF2 protein. In turn, the ensuing nuclear accumulation of NRF2 induces genes, including those that increase antioxidant status (e.g. *SLC7A11, GCLC, GCLM,*

GPX2, *HMOX1*, *PRDX1*, *SRXN1*, *TXN1* and *TXNRD1*), increase detoxification capacity (e.g. *AKR1B10*, *AKR1C1*, *AKR1C2*, *AKR1C3* and *NQO1*) and reprogram metabolism (e.g. *G6PD*, *IDH1*, *ME1*, *PGD*, *TALDO1* and *TKT*).

(Image created by Rumen V. Kostov, University of Dundee).