Cancer-Derived Mutations in KEAP1 Impair NRF2 Degradation but not Ubiquitination

Bridgid E. Hast1, Erica W. Cloer1, Dennis Goldfarb4, Heng Li5, Priscila F. Siesser1, Feng Yan1, Vonn Walter2, Ning Zheng5, D. Neil Hayes3, and Michael B. Major1,2,4

Abstract

NRF2 is a transcription factor that mediates stress responses. Oncogenic mutations in NRF2 localize to one of its two binding interfaces with KEAP1, an E3 ubiquitin ligase that promotes proteasome-dependent degradation of NRF2. Somatic mutations in KEAP1 occur commonly in human cancer, where KEAP1 may function as a tumor suppressor. These mutations distribute throughout the KEAP1 protein but little is known about their functional impact. In this study, we characterized 18 KEAP1 mutations defined in a lung squamous cell carcinoma tumor set. Four mutations behaved as wild-type KEAP1, thus are likely passenger events. R554Q, W544C, N469fs, and P318fs, and G333C mutations attenuated binding and suppression of NRF2 activity. The remaining mutations exhibited hypomorphic suppression of NRF2, binding both NRF2 and KEAP1. Proteomic analysis revealed that the R320Q, R470C, G423V, D422N, G186R, S243C, and V155F mutations augmented the binding of KEAP1 and NRF2. Intriguingly, these “super-binder” mutants exhibited reduced degradation of NRF2. Cell-based and in vitro biochemical analyses demonstrated that despite its inability to suppress NRF2 activity, the R320Q “superbinder” mutant maintained the ability to ubiquitinate NRF2. These data strengthen the genetic interactions between KEAP1 and NRF2 in cancer and provide new insight into KEAP1 mechanistics. Cancer Res; 74(3); 808–17. © 2013 AACR.

Introduction

In contrast to the mutational clustering seen in oncogenes, where a few residues are frequently affected, mutations in tumor suppressor proteins typically lack focal enrichment. This creates uncertainty about the impact of specific mutations on protein function; mutations may be phenotypically silent "passenger" events, they may result in a spectrum of hypomorphs, or produce a functionally dead protein. Cataloged associations between specific cancer genotypes and protein function will instruct many principles of cancer biology and oncology, including patient stratification for targeted therapy.

The Cancer Genome Atlas (TCGA) recently reported the characterization of 178 squamous cell lung carcinomas (SQCC), revealing at least 10 recurrently mutated genes.
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an increase in cancer cell proliferation (6). Although comprehensive data are not complete, several studies have reported that NRF2 activity correlates with poor prognosis and chemotherapy resistance (7–10).

The now established importance of KEAP1–NRF2 in promoting cancer cell growth and survival underscores the need to elucidate how cancer evolution leads to pathway activation. Several mechanisms are easily recognized from cancer genomic studies: activating mutations in NRF2 free it from KEAP1 association (11), copy number amplifications of the NRF2 genomic locus increase protein expression, and KEAP1 promoter hypermethylation decreases its mRNA and protein expression (12, 13). What remains uncertain is which somatic mutations within KEAP1 affect its function, to what degree do they impact function, and mechanistically how its function is compromised. Recent efforts from several groups have identified correlations between cancer genotype and phenotype, and these findings may have a significant impact on clinical interventions (14–18). With these concepts in mind, we functionally tested and biochemically characterized KEAP1 mutations found within lung SQCC. Our data connects can-...
assay, wild-type KEAP1 or the R320Q mutant was mixed with recombinant human E1, UbcH5, CUL3-RBX1, ubiquitin, and GST-tagged NRF2 NEH2 domain (GST-NRF2-NEH2) in buffer containing 40 mmol/L Tris-HCl pH 8.0, 5 mmol/L MgCl2, 2 mmol/L DTT, and 4 mmol/L ATP. Ubiquitination was carried out at 37°C and the products were analyzed by Western blot with anti-GST antibody.

**Immunostaining**

HEK293T cells were cotransfected with the indicated plasmids and plated on 10 μg/ml fibronectin-coated coverslips. Cells were fixed in 4% paraformaldehyde in cytoskeletal buffer for 15 minutes, and coverslips were mounted to slides using the Prolong Gold antifade reagent (Molecular Probes). Images were acquired using a Zeiss LSM5 Pascal Confocal Laser Scanning Microscope equipped with ×63/1.42 Oil PlanApo objective lenses.

**Affinity purification and mass spectrometry**

For streptavidin and FLAG affinity purification, cells were lysed in 0.1% NP-40 lysis buffer. Cell lysates were incubated with streptavidin or FLAG resin and washed 5 times with lysis buffer. The precipitated proteins were trypsinized directly on beads using the FASP Protein Digestion Kit (Protein Discovery).

**Protein identification, filtering, and bioinformatics**

All raw data were converted to mzXML format before a search of the resultant spectra using SocrerFTR-MS/SEQUEST (build 4.0.4; Sage N Research) and the Transproteomic Pipeline (TPP v4.3.1). Data were searched against the human UniprotKB/Swiss-Prot sequence database (Release 2011_08) supplemented with common contaminants, that is porcine (Swiss-Prot P00761) and bovine (P00760) trypsin, and further concatenated with its reversed copy as a decoy (40,494 total sequences). Search parameters used were a precursor mass tolerance of 0.5 amu, semitryptic digestion, a static carbamidomethyl cysteine modification, variable methionine oxidation, and variable phosphorylation of serines, threonines, and tyrosines. False discovery rates (FDR) were determined by ProteinProphet and minimum protein probability cutoffs resulting in a 1% FDR were selected individually for each experiment. ProteinProphet/ProteinProphet results for each affinity purification and mass spectrometry (APMS) experiment were stored in a local ProHits database. To determine an interacting protein’s abundance relative to wild type (WT), prey spectral counts were bait normalized by dividing by the bait spectral count, followed by calculating the number of standard deviations from wild type (similar to a Z-score), where the SD was computed for each prey individually. Unfiltered data and spectral count normalizations are provided as Supplementary Table S2.

**Results**

**Connecting cancer-derived KEAP1 mutations with NRF2 activity**

A search of the literature and public domain revealed 213 somatic mutations in KEAP1, observed across 17 cancer types and multiple cell lines (Supplementary Table S1). Mapping these mutations onto the KEAP1 primary amino acid sequence revealed a mostly uniform distribution of affected residues (Fig. 1A). The distribution of mutations specifically found in squamous cell lung carcinoma further reiterated the lack of a “mutation cluster region” (Fig. 1A, blue ovals). Of the 18 mutations found in lung SQCC, only 2 mutations resulted in a truncated protein product (N469fs and P318fs). The remaining 16 missense mutations included the addition of 3 new cysteine residues (G333C, W544C, and S243C), which might alter KEAP1 reactivity to electrophilic agents. One mutation, V155F occurred in 2 separate tumors, and interestingly, none of the mutations in KEAP1 were in residues that directly interface with NRF2 (20). Given the importance of KEAP1-NRF2 signaling in cancer and our inability to predict the functional consequences of KEAP1 mutation, we cloned and comparatively evaluated each of the 18 lung SQCC mutations.

To test whether cancer-derived mutations in KEAP1 affect NRF2-driven transcription, we used an engineered reporter system, wherein the luciferase gene is expressed in a NRF2-dependent manner. Ectopic expression of wild-type KEAP1 suppressed NRF2-dependent luciferase expression in HEK293T cells (Fig. 1B). By comparison, the KEAP1 mutants displayed variable suppression of NRF2-driven transcription. Specifically, L231V, S224Y, P318L, and R71L suppressed NRF2 as well as wild-type KEAP1; these genotypes represent possible passenger mutations within KEAP1. By contrast, N469fs, P318fs, and G333C exhibited a near-null phenotype. Most surprisingly, of the 18 mutants examined, 11 retained partial ability to suppress NRF2-driven transcription. To further validate these data, we tested the panel in the lung adenocarcinoma cell line A549, which express mutant and inactive KEAP1G333C, and in Keap1 knockout MEFs. In all 3 cell lines tested, which vary in KEAP1 genotype, we observed a consistent pattern of KEAP1-mediated NRF2 suppression (Fig. 1C and D).

At its core, this work sought to isolate and functionally annotate specific KEAP1 genotypes so that clinical correlations and predictions might be drawn from genome sequence data alone. As such, we tested whether the relative activities of each KEAP1 mutant correlated with the expression of 15 NRF2 target genes within the lung SQCC TCGA cohort (1, 4). Comparing luciferase activity (Fig. 1B–D) to the NRF2 transcriptional gene signature, we found that mutants that suppress like wild-type KEAP1 associate with decreased NRF2 activity, whereas mutants unable to completely suppress NRF2 correlate with increased NRF2 target gene expression (P = 0.049; 2-sided Wilcoxon rank sum test; Supplementary Fig. S1A). Any attempt to further segregate mutations based on luciferase activity did not show a statistically significant correlation in the patient data.

**Biochemical characterization of the KEAP1 mutants**

Next, we sought molecular insight into how specific mutations differentially impacted KEAP1 function. First, we determined whether the mutants expressed at levels similar to wild-type KEAP1, as nonsynonymous mutations often impair protein folding to decrease protein stability. Transient
expression from plasmid DNA indicates that the majority of KEAP1 mutants expressed at levels similar to wild-type protein (Fig. 2A and Supplementary S1B). Further study is required to determine if the reduced expression of mutants R554Q, W544C, N469fs, P318fs, G480W, and G333C is because of altered protein or mRNA stability. To extend these data, the subcellular localization of KEAP1 and each KEAP1 mutant was evaluated in HEK293T cells; all mutants exhibited a localization pattern indistinguishable from wild-type KEAP1 (Supplementary Fig. S1C).

KEAP1 functions as a critical sensor of oxidative stress, wherein multiple cysteine residues act as bioensors for ROS and xenobiotic molecules (11, 21, 22). In cells, KEAP1 is thought to exist as a homodimer, creating a 2:1 stoichiometry with the NRF2 substrate. Following cysteine modification, either by reactive oxygen species or by electrophilic agents like tert-butylhydroquinone (tBHQ), a conformational change within the KEAP1 homodimer creates an SDS-resistant form that is readily visualized under denaturing electrophoresis (23, 24).

Mutations in KEAP1 positively correlate with increased NRF2 activity. A, probability density function of KEAP1 mutations were approximated using kernel density estimation. Lung SQCC mutations examined in this study are annotated above; all mutations from the public domain are shown below and in Supplementary Table S1. B, HEK293T cells, Keap1 knockout MEFs, and A549 cells were transiently transfected with the indicated plasmids along with constitutively expressed Renilla luciferase and the NQO1 promoter driving Firefly luciferase. Cells were lysed and Firefly luciferase values were normalized to the luciferase activity of the Renilla control. Error bars represent SD from the mean over 3 biologic replicates (NTR, N-terminal region; BTB, tramtrack and bric-a-brac; CTR, C-terminal region; IVR, intervening region).

Together, these data suggest that with the exception of KEAP1 mutants interacting with CUL3 (Supplementary Fig. S2A). Immunoprecipitation and Western blot analysis revealed that all of the KEAP1 mutants interact with CUL3 (Fig. 2C). Further analysis is needed to determine if the subtle differences in CUL3 binding reflect differential affinities or expression variability (Fig 2C, compare lanes 15, 16, and 19). Next we determined if the KEAP1–NRF2 association was maintained among the mutants. Western blot analysis of immunopurified KEAP1 and mutant KEAP1 protein complexes showed that the R554Q, W544C, N469fs, G480W, G333C mutants failed to bind NRF2 (Fig. 3A and Supplementary Fig. S2B and S2C). Surprisingly, however, the remaining 13 KEAP1 mutants retained NRF2 binding. Together, these data suggest that with the exception of R554Q, W544C, N469fs, P318fs, and G333C, SQCC-derived KEAP1 mutants maintain their ability to bind both NRF2 and CUL3.

Mass spectrometry–based proteomic analysis of KEAP1 revealed 42 high confidence–associated proteins (4). To gather a global perspective of how the mutations affect KEAP1 protein interactions, we performed 2 experiments. First, we tested the association of 7 high confidence–interacting proteins by immunoprecipitation and Western blot analysis. The data show a distinct pattern among the KEAP1 mutants; those that do not bind NRF2 fail to bind several of the known interactors, including SLK, AMER1 (WTX), MCM3, DPP3, and IKKβ (IKK-β; Fig. 3A and Supplementary Fig. S2C). Interestingly, all of these proteins contain an ETGE motif (4). Two mutations, G480W and S224Y, show decreased
binding to SLK, MCM3, and DPP3 as compared with NRF2 (Fig. 3A, lanes 8 and 15). Second, we used affinity purification and shotgun mass spectrometry to define and compare the protein interaction network for wild-type KEAP1 and the following mutants: R554Q, R320Q, R470C, G480W, G423V, D422N, G186R, S243C, and V155F (Fig. 3B and Supplementary Table S2). The unbiased proteomic screens confirm the Western blot analysis results and further expand the pattern of altered protein interactions.

A class of KEAP1 mutants with increased NRF2 binding

We were particularly intrigued with a subset of mutants that consistently bound more NRF2 than wild-type KEAP1 (Fig. 3A, lanes 3, 5, 6, 9, 14, 16, 19, and Supplementary Fig. S2C). We collectively refer to these mutants as the "superbinders," although relative protein affinity is not meant to be inferred. The super binder mutants include R320Q, R470C, G423V, D422N, G186R, S243C, and V155F. Increased abundance of NRF2 within each super binder protein complex was confirmed by immunoprecipitation and quantitative Western blot analysis (Supplementary Fig. S3A and S3B). In addition, label-free mass spectrometry comparing wild-type KEAP1 and two super binder mutants (R320Q and R470C) showed an increased abundance of NRF2 as compared with wild-type KEAP1; based on spectral counts, R320Q and R470C bound 3.3- and 3.2-fold more NRF2 than wild-type KEAP1, respectively (Fig. 3B and Supplementary Table S2). For comparative purposes, we performed quantitative proteomic analysis on 2 nonsuper binder mutant proteins: R554Q, which cannot bind NRF2 and G480W, which binds NRF2 similarly to wild type (Fig. 3B).

Despite an increased level of associated NRF2, the super binder mutants were unable to suppress NRF2-mediated transcription of an artificial reporter gene (Fig. 1B–D). To confirm this using endogenous metrics of NRF2 activity, HEK293T cells, H2228 cells, or A549 cells were transiently transfected with wild-type KEAP1 or the super binder mutants before Western blot analysis of NRF2 and the NRF2 target gene HMOX1. Transient expression of each super binder strongly increased the levels of NRF2 and HMOX1 in the H2228 and A549 cell lines.
KEAP1 mutations that show significant ubiquitination but not degradation. KEAP1 superbinder mutants facilitate NRF2 stabilization and elevated levels within the nuclear compartment (Fig. 4C and Supplementary Fig. S3C).

Subcellular fractionation of the HEK293T cells further revealed that KEAP1 superbinder expression increased the levels of NRF2 within the nuclear compartment (Fig. 4C and Supplementary Fig. S3C).

KEAP1 superbinder mutants facilitate NRF2 ubiquitination but not degradation

Our functional and biochemical examination revealed 7 KEAP1 mutations that show significantly impaired ability to suppress NRF2, but yet unexpectedly bind more NRF2 than wild-type KEAP1. To gain further insight, we evaluated NRF2 protein turnover and ubiquitination following KEAP1 superbinder expression. Using a cycloheximide (CHX) pulse-chase approach, NRF2 protein half-life was evaluated in HEK293T cells stably expressing wild-type KEAP1 or G480W (Supplementary Fig. S3D and Fig. 5A). The increased NRF2 stability occurred as a result of binding R320Q or R470C, as unbound NRF2 in the flow-through eluate showed elevated levels but dynamic turnover (Fig. 5B, compare flow-through to KEAP1 immunopurification). Together, these data suggest that the superbinder mutations within KEAP1 result in the stabilization of KEAP1-associated NRF2 and elevated levels of free NRF2, although the free NRF2 is still subject to dynamic turnover.

Figure 3. KEAP1 mutant proteins exhibit differential binding to interacting proteins. A, HEK293T cells expressing the indicated KEAP1 mutants were treated with 10 μM MG132 for 1 hour, followed by FLAG immunopurification. Protein complexes of the FLAG-tagged mutants were analyzed by Western blot for the indicated proteins. Red lines indicate KEAP1 mutants that were analyzed by mass spectrometry as indicated in B. B, APMS experiments were performed via affinity purification of streptavidin-tagged KEAP1 mutants from stable HEK293T cells followed by mass spectrometry analysis of the bound proteins. Colors represent normalized spectral counts—semiquantitative values that reflect protein abundance—from the APMS experiments. Proteins displayed are previously identified high-confidence KEAP1 interactors.

Figure 4. Expression of KEAP1 superbinder mutants enhances nuclear localization of NRF2. A, H2228 cells were cotransfected with the indicated FLAG-tagged KEAP1 mutant plasmid or negative control Venus-NPM1, and NRF2 plasmid. Cells were lysed in RIPA buffer and analyzed by Western blot for the indicated proteins. B, A549 cells were transiently transfected with the indicated KEAP1 mutants or Venus-NPM1, and protein lysates were analyzed by Western blot for the indicated endogenous (NRF2, MEK1/2, H3) and ectopically expressed proteins.
Given the increased NRF2 association and protein stability, we hypothesized that R320Q and other superbinder mutants impair NRF2 ubiquitination. To test this, we performed 2 complementary experiments to evaluate NRF2 ubiquitination by wild-type KEAP1 or the R320Q superbinder. First, Western blot analysis of immunoprecipitated NRF2, after denaturation, showed robust ubiquitination by both wild-type KEAP1 and R320Q (Fig. 5C). Second, we performed in vitro ubiquitination reactions using purified proteins (Fig. 5D). Remarkably, both experimental approaches demonstrate that wild-type KEAP1 and R320Q ubiquitinate NRF2.

Discussion

With some latitude, we can classify the 18 KEAP1 mutations into 3 classes. First, the L231V, S224Y, P318L, and R71L mutations...
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Mutations did not impact the KEAP1–NRF2 association or the suppression of NRF2 activity. These mutations likely represent passenger events within KEAP1, at least with respect to NRF2. Second, and not surprisingly, the frame shift mutations N469fs and P318fs, as well as G333C, R554Q, and W544C did not bind NRF2 and did not or weakly suppressed NRF2-mediated transcription. These genotypes represent null or near-null alleles. Third, the remaining 9 mutations fell within a hypomorphic phenotypic range, with suppression occurring between 30% and 60% of the wild-type KEAP1. Biochemically, the hypomorphic mutants displayed either reduced NRF2 binding or surprisingly, increased binding (the superbinders).

Mutations in tumor suppressor genes often results in complete loss of protein expression or the expression of a truncated protein product (25). It is therefore intriguing to consider why KEAP1 is rarely lost through genomic deletion, despite being located between the SMARCA4 and STK11 tumor suppressor genes on 19p (cBioPortal). A number of loosely connected observations raise the possibility that KEAP1 may exert cancer-relevant functions that extend beyond regulation of oxidative stress and NRF2. First, we found that many KEAP1 mutations result in a hypomorphic phenotype, rather than a genetic null. Second, in general, these hypomorphic mutations do not affect the global KEAP1 protein interaction network, suggesting that some KEAP1 protein interactions are retained in the absence of NRF2 suppression (Fig. 3B and Supplementary Table S2). Indeed, KEAP1-associated proteins regulate a number of disparate cellular processes, including cell cycle, migration, and apoptosis (4, 26–34). Third, although the presence and importance of NRF2-independent KEAP1 functions remain unknown, we and others have established that several KEAP1-interacting proteins drive NRF2 activation via a competitive binding mechanism (4, 19, 35–37). Previously, we found that hypomorphic KEAP1 mutants can be further inactivated by the ETGE-containing competitive binding protein, DPP3 (4). Coupled with the observed overexpression of DPP3 in lung squamous cell carcinoma, these observations suggest that from the perspective of cancer cell fitness, the presence of a hypomorphic KEAP1 mutation may be more valuable than a null mutant.

The most surprising and perhaps exciting discovery we observed was the identification of the superbinders—those that do not suppress NRF2-mediated transcription, exhibit enhanced binding to NRF2, and facilitate NRF2 ubiquitination. Three points of discussion are appropriate. First, by what mechanism could the superbinder mutations affect NRF2 stability? Several possibilities exist, including an increased affinity between KEAP1 and NRF2 as a means to suppress substrate turnover. Analogously, the expression of a superbinder variant SH2 domain antagonizes epidermal growth factor signaling via competitive inhibition (38). That said, although studies are ongoing, the lack of a focal enrichment within the tertiary structure casts some doubt on this possibility (Fig. 5E). Cullin ring E3 ubiquitin ligases cycle through an active and inactive state, and this neddylation-dependent transitioning is required for substrate turnover. A second possibility is that the superbinder mutations simply slow the rate of CUL3 neddylation. Finally, proteasome-mediated substrate degradation requires several steps, including recognition, unfolding, translocation, and deubiquitination before proteolysis (39). The striking observation that the enhanced NRF2-binding class of KEAP1 mutants ubiquitates NRF2 suggests that the mutations functionally hinder one of the steps before proteolysis, but after ubiquitination. Here, immediate questions include whether the superbinder mutations affect the ubiquitin chain linkage on NRF2 or whether they perturb the interaction of KEAP1 with the proteosome. All 3 of these putative mechanisms to describe the superbinder phenotype would inactivate KEAP1 and stabilize NRF2 in a manner consistent with the widely accepted "saturation model" (22). Importantly, as the KEAP1 mutants described in this study exhibit hypomorphic phenotypes, the superbinders could represent a novel mechanism cancer cells use to enhance cellular fitness without compromising all cellular functions of multifunctional proteins.

Second, it is now widely accepted that elevated levels of NRF2 are associated with enhanced cell viability in several tumor types (7, 10, 40–42). Although we show that “superbinder” mutations result in NRF2 transcriptional activation, further studies are required to determine whether this KEAP1 mutant class is capable of enhancing cancer cell fitness in vivo, and whether that depends upon prolonged activation of NRF2. In addition, given emerging evidence identifying other putative KEAP1 substrates in cancer-relevant pathways, such as IKK-β within NF-κB signaling (5, 43), investigating how—if at all—superbinder mutations impact these proteins could also have clinical significance. Looking at the full set of KEAP1 mutant tumors and the expression of 15 NRF2 target genes, a marginal but statistically significant difference was observed between phenotypically “silent” KEAP1 mutations and mutations that suppress KEAP1-driven NRF2 degradation (Supplementary Fig. S1A). Our attempts to more precisely correlate KEAP1 genotype with the cell-based phenotypic scoring failed to reach statistical significance. This is not surprising given the multitude of signaling and metabolic inputs that control KEAP1.

Third, from a structural perspective, we noted weak correlation between the tertiary position of a mutation and whether the mutation produced a KEAP1 superbinder (Fig. 5E). Although speculative, the superbinder mutations seem to be localized at positions that might orient the relative position of intervening region (IVR) and KELCH domains; experiments testing this model are ongoing. Intriguingly, of the 181 missense mutations reported in KEAP1, 6 directly target the R320 superbinder residue, making it the most commonly affected amino acid in KEAP1 (Fig. 1A). Beyond the superbinder mutations, mapping all SQCC 19 mutations onto the KEAP1 structure failed to reveal a discernible pattern. Likewise, side-chain biochemistry for the mutations varies widely, including those within the superbinder class. Cysteine reactivity depends upon the local chemical microenvironment, which is largely dictated by the surrounding amino acids in a protein tertiary structure. Hence, for a cysteine-dependent biosensor like KEAP1, oncogenesis may partially suppress KEAP1 activity by selecting for mutations that add cysteines (S245C, G335C, R470C) or that reduce the relative pKa of existing cysteines, making them more sensitive to electrophilic attack (44). Clearly, spatial constraints preclude the random addition of cysteines as a means to increase the reactivity of KEAP1 to oxidative stress.
New cancer-derived cysteines of functional importance would occupy specific localizations within the folded protein. By extension of this idea, cancer-derived mutations that create "hyperactive" cysteines within KEAP1 would be expected to produce a hypomorphic phenotype, as we have observed. Further study is needed to support these ideas, perhaps through the functional and biochemical characterization of the other 213 cancer-derived mutations in KEAP1. To this end, medium-throughput functional analysis is facilitated by Gateway-based cloning, outsourced mutagenesis, and a strong pathway-specific transcriptional reporter. The resulting data may better enable predictions of genotype–phenotype relationships. However, based on the KEAP1 data presented here, it is not yet possible to derive functional conclusions from the location of a mutation or the type of residue substitution.

In summary, we describe the functional and biochemical characteristics of 18 mutations in the E3 ligase adaptor protein KEAP1, which were found in patient-derived lung cancers. We show that although most of these mutations maintain similar protein interactions to wild-type KEAP1, all but 4 exhibit hypomorphic or null activity with respect to suppression of NRF2-mediated transcription. Intriguingly, a subset of these mutations exhibit enhanced binding to NRF2 despite an inability to suppress NRF2 activity. Functional analysis of one of these mutants, R320Q, revealed that these mutants are still able to ubiquitinate NRF2, but seem to be unable to facilitate its degradation. Further studies are required to elucidate the mechanism of this class of KEAP1 mutations, including how they interact with the proteasome, as well as whether these mutants enhance viability of cancer cells via prolonged activation of NRF2.

References

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Bridgid E. Hast, Erica W. Cloer, Dennis Goldfarb, et al.


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