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COMMENT

EMSY stabilization in *KEAP1*-mutant lung cancer disrupts genome stability and type I interferon signaling

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Perturbed proteostasis is an emerging hallmark of cancer, exhibiting aberrations in protein degradation by the ubiquitin-proteasome system (UPS) [1–3]. Numerous studies provide evidence that dysregulations in the activity of ubiquitin E3 ligases, which mediate the selective ubiquitination and subsequent degradation of critical cellular proteins, are associated with different aspects of tumorigenesis, cancer progression, tumor immune infiltration, and response to therapy [3]. Thus, understanding the molecular mechanisms that underlie such aberrations, and discovering the protein targets whose controlled degradation is perturbed in cancer, constitute an attractive area for cancer research. Such cancer-associated alterations in protein stability are becoming therapeutically targetable by the recent technologies in the targeted protein degradation (TPD) field and precision medicine [4, 5].

One exemplar case of a dysregulated E3 ligase in cancer is the recurrent inactivating mutations in a ubiquitin E3 ligase called KEAP1 (kelch-like ECH-associated protein 1) in non-small cell lung cancer (NSCLC) [6, 7]. *KEAP1*, which encodes for a substrate receptor of a CUL3-RING ubiquitin ligase (CRL3), is considered to be among the top lung cancer drivers. Specifically, this tumor suppressor was shown to be mutated in about 15% of NSCLC patients. Further, *KEAP1*-mutated NSCLC was previously shown to exhibit immune evasive features, such as lower T cell infiltration and resistance to current immune checkpoint inhibitors [8, 9]. Yet, the underlying mechanisms and the identity of the aberrantly degraded substrates in *KEAP1*-mutated NSCLC, remain poorly understood.

Under physiological conditions, KEAP1 together with its well-established substrate, the transcription factor NRF2, are the master regulators of cellular oxidative homeostasis [7]. However, activating mutations of *NRF2* in NSCLC do not share the same properties with inactivating mutations of *KEAP1*, which suggests that other unknown substrates might underlie *KEAP1*-mediated tumor-promoting properties and immune evasiveness. In the elegant work published by the Pagano group, Marzio et al. [10] revealed that deregulation of KEAP1 attenuates anti-tumor immunity and DNA damage repair (DDR) through the protein stabilization of EMSY, a pro-oncogenic chromatin remodeler and BRCA2 inhibitor. The authors showed that *KEAP1* deficiency induces an EMSY-dependent "BRCAness phenotype [11]" characterized by defects in homologous recombination repair (HRR) and sensitivity to PARP inhibitors (PARPis). Furthermore, they found that the accumulation of EMSY, which is observed in *KEAP1*-deficient tumors, negatively regulates the type I interferon response, suppressing innate immunity and promoting lung cancer immune evasion (Fig. 1).

By evaluating the tumor mutational burden (TMB) distribution across the major drivers of NSCLC, Marzio et al. [10] set out to uncover a possible association between *KEAP1*-mutant tumors, TMB and impairment in the pathways involved in DDR. Indeed, the authors found that *KEAP1*-mutated NSCLCs exhibit a higher TMB based on data from the cancer genome atlas (TCGA). Further, by generating a knockout to *Keap1* on the background of the KP murine NSCLC model (*K-ras* mutant, *Trp53* null genotype), they could confirm that loss of *KEAP1* significantly increased the mutational load by whole genome sequencing. Next, the authors defined the *KEAP1*-mutated NSCLCs cells and identified a BRCAness phenotype based on aberrant DDR, mainly in HRR. Previous studies have identified a synthetic lethality between HRR defects and poly ADP-ribose polymerase (PARP) inhibitors [11–14]. Thus, using cell-based systems, human PDXs, and NSCLC mouse model of tumorigenesis, Marzio et al. [10] examined whether *KEAP1*-mutated tumors display sensitivity to PARP inhibition, in vivo. Notably, the effect of the PARPi was significant and observed only in the absence of *KEAP1* and was not significant on the background of the KP line.

Since NRF2 is the best characterized substrate of KEAP1, *KEAP1*-mutant tumor phenotypes are often attributed to the upregulation of NRF2. However, in their study, NRF2 activation, or expression of a non-degradable NRF2 did not result in sensitization to PARPi, which suggested that another target of KEAP1 may be at play. To identify this putative target, the authors utilized a proteomic approach combined with biochemical studies and discovered that KEAP1 interacts with the transcriptional repressor EMSY. Since EMSY was known to interact with BRCA2 and to induce BRCAness upon its overexpression [15, 16], the authors sought to explore whether it is indeed the relevant substrate for KEAP1 responsible for said phenotype. Indeed, they could show that EMSY is targeted to degradation by the proteasome following KEAP1-mediated ubiquitination. Conversely, inactivating mutations of *KEAP1* resulted in EMSY accumulation in murine cell models as well as human NSCLCs. Importantly, the identification of a degron sequence in *EMSY* that was found to be critical for its binding to KEAP1 and its consequent degradation has allowed the researchers to elegantly demonstrate that EMSY mediates the BRCAness phenotype of *KEAP1* mutant tumors. Further,

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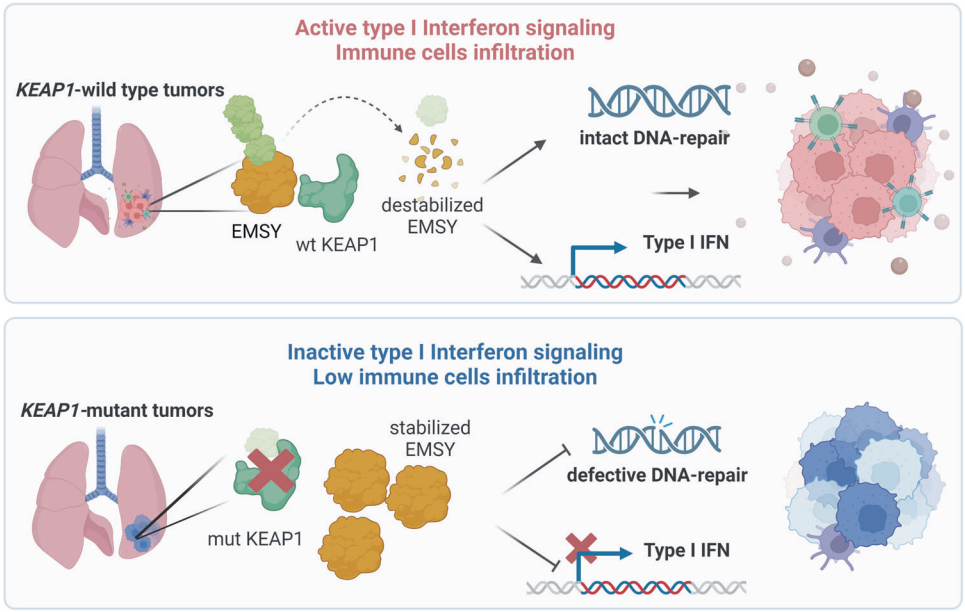

Fig. 1 Stabilization of EMSY in *KEAP1*-mutant NSCLC results in the inactivation of type I interferon signaling, reduction in DNA repair, and lowering of immune cell infiltration. The figure was created with BioRender.com.

overexpression of a non-degradable EMSY version was sufficient to result in sensitization to PARPi and promote genomic instability by disrupting the HRR pathway. Moreover, the depletion of EMSY in *KEAP1*-mutated NSCLCs was sufficient to revoke their BRCAness phenotype.

Next, the authors found that the activation of the type I interferon response is compromised in *KEAP1*-mutant tumors, underscoring the previous role of EMSY [17, 18], as a transcriptional repressor of interferon-stimulated genes (ISGs) to control antiviral immunity. The authors then showed that EMSY-mediated suppression of type I interferon signaling promotes cancer immune evasion. Specifically, they found that EMSY deficiency impairs tumor growth in a non-cell autonomous manner. Namely, depletion of *EMSY* in *KEAP1*-mutated tumors, or control tumors injected into NSG immune incompetent mice did not result in affecting tumor growth whereas *EMSY* depletion in *KEAP1*-deficient tumor cells injected into immune-competent mice significantly reduced tumor growth. Finally, the authors showed that EMSY deficiency impairs tumor growth via signaling of type I interferon in the tumor microenvironment (TME), as depletion of *EMSY* in *KEAP1*-mutated tumor cells injected into *IFNAR1* KO mice did not significantly reduce tumor growth. In addition, the authors demonstrated that the immune-suppressive effect of *KEAP1*-mutation in the tumor is EMSY-dependent and mediated through increased CD206 + M2 pro-tumorigenic macrophages, and lower CD8. As the cGAS/STING pathway is a master regulator of type I interferon response in TME [19], the authors used an FDA-approved STING agonist (STINGa) to counteract the pro-oncogenic activity of EMSY in the tumor and stimulate anti-tumor immunity in the TME. It remains to be elucidated whether the observations are explained by a reversal of the type I interferon repression in either the cancer cells themselves, or by a direct effect on the immune cells, or both.

Importantly, when combined with PARPi, STINGa synergistically reduced the tumor weight of *KEAP1*-mutant tumors supporting the rationale for combining these two therapies in NSCLC tumors with inactivating mutations in KEAP1. Although the treatment did not completely abolish tumor growth, the combination led to an impressive effect on *KEAP*-mutated tumors, possibly expanding the therapeutic potential for NSCLC patients harboring *KEAP1* mutations.

Several interesting questions arise from the findings by Marzio et al. [10]. For example, since EMSY is a transcriptional repressor that is part of several chromatin-bound complexes, it will be intriguing to examine whether a specific EMSY-containing remodeling complex is involved in the suppression of type I interferon response. Further, given the established interaction between BRCA2 and EMSY, is BRCA2 also playing a transcriptional role in *KEAP1*-mutant tumors? And finally, does KEAP1 also directly regulate components of the adaptive immune response?

In summary, the work described by Marzio and colleagues uncovered the molecular mechanism by which *KEAP1* mutations promote immune evasion, namely by suppression of the type I interferon response in the TME. Specifically, it highlights the crucial role of innate immunity in tumor surveillance and immunological memory. As such, it established the basis for examining the potential effect of cGAS/STING modulators in combination with immunotherapy, as a novel path for invigorating anti-tumor immunity in patients bearing *KEAP1* mutations in NSCLC and other cancer types.

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AUTHOR CONTRIBUTIONS

DS and YM wrote the paper and prepared the figure.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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