CELL CYCLE
2017, VOL. 16, NO. 4, 297–298
http://dx.doi.org/10.1080/15384101.2016.1232087

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EDITORIALS: CELL CYCLE FEATURES

ROS in translation: Chink in the armor

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ARTICLE HISTORY Received 23 August 2016; Accepted 27 August 2016

KEYWORDS cysteine thiols; NRF2; protein synthesis; pancreatic cancer; redox homeostasis

Pancreatic ductal adenocarcinoma (PDA) holds the most dismal prognosis among the common malignancies and is considered to be largely incurable. In spite of extensive efforts in preclinical and clinical science, the survival rate of pancreatic cancer has not improved substantially over the past 40 y. An activating mutation of KRAS is the most common genetic perturbation found in PDA, occurring in 90–95% of the cases. Despite the identification of a clear driver oncogene, clinically actionable strategies to target KRAS have not yet been identified. A deeper understanding of the molecular events downstream of KRAS is therefore critical for the development of new therapeutic interventions for this deadly disease.

Our laboratory previously identified the transcription factor Nuclear factor erythroid-derived 2-like 2, NFE2l2/NRF2 to be up-regulated upon oncogenic KRAS expression and supports pancreatic tumor initiation. The NRF2 transcriptional program is central to the maintenance of cellular redox homeostasis and has been the focus of much research to understand the impact of oxidative stress in tumorigenesis. In our recent publication,[1] we demonstrated that NRF2 is a critical factor for pancreatic tumor maintenance through preserving the functional integrity of the protein synthesis machinery.

Using a pancreatic organoid culture system that we developed recently,[2] we observed that human PDA organoids were more sensitive to suppression of NRF2 than normal counterparts, presenting a case of synthetic lethality not previously recognized for this transcription factor. NRF2 deletion in murine PDA organoids led to elevated levels of reactive oxygen species (ROS) and decreased cell proliferation. The chemical properties of cysteine thiol groups render this amino acid exquisitely sensitive to changes in cellular levels of ROS, thus suggesting reactive-cysteine-containing proteins to be potential candidates to carry out redox-sensitive effector functions of NRF2. To decipher the role of NRF2 in pancreatic tumor maintenance, we devised a highly sensitive proteomic method to quantify changes in the cysteine proteome. Using this approach, we identified cysteines on translational regulatory proteins to be explicitly oxidized in NRF2-deficient PDA organoids. Using a variety of biochemical approaches, we confirmed that both cap-dependent and cap-independent mRNA translation were impaired in NRF2-deficient pancreatic cancer cells, and can be rescued upon supplementation with antioxidants. Thus, NRF2 armors the cancer cell translation apparatus against insults from ROS to support PDA growth and survival (Fig. 1).

Our cysteine proteomic approach has provided new perspectives into the biochemical regulation of the translation apparatus. While the control of protein synthesis at the initiation step is well recognized to play an important role, regulation at the elongation step has received less attention. Elongation is a highly energy demanding process that relies on only 3 factors. Among these factors, elongation factor 2 (eEF2), which catalyzes the ribosomal movement along the mRNA, is a target of several distinct regulatory signaling pathways, and may represent a central regulator of the elongation process. eEF2 promotes the GTP-dependent translocation of the nascent polypeptide chain from the A-site to the P-site of the ribosome and can be regulated by various post-translational modifications including phosphorylation, acetylation and ADP-ribosylation. Through studying the cysteine proteome, we identified cysteine 693, a residue conserved between mice and humans, to be a functional and novel redox switch on eEF2. This is consistent with an earlier report demonstrating sulfenylation on this residue.[3] Interestingly, both eEF2 ADP-ribosylation and activity have been shown to be redox dependent.[4] It remains to be determined how oxidation of C693 operates in conjunction with other post-translational modifications on eEF2 to mediate protein activity.

Cancer cells require an aberrantly activated translational state for survival, thus allowing the targeting of the translation machinery in neoplastic cells while limiting toxicity for normal cells. Components of the translational machinery, such as eIF4E/4EBP1, and signal transduction pathways involved in the regulation of translation initiation, such AKT

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Feature to: Chio II, et al. NRF2 Promotes Tumor Maintenance by Modulating mRNA Translation in Pancreatic Cancer. Cell 2016; 166(4):963-76; PMID: 27477511; http://dx.doi.org/10.1016/j.cell.2016.06.056.

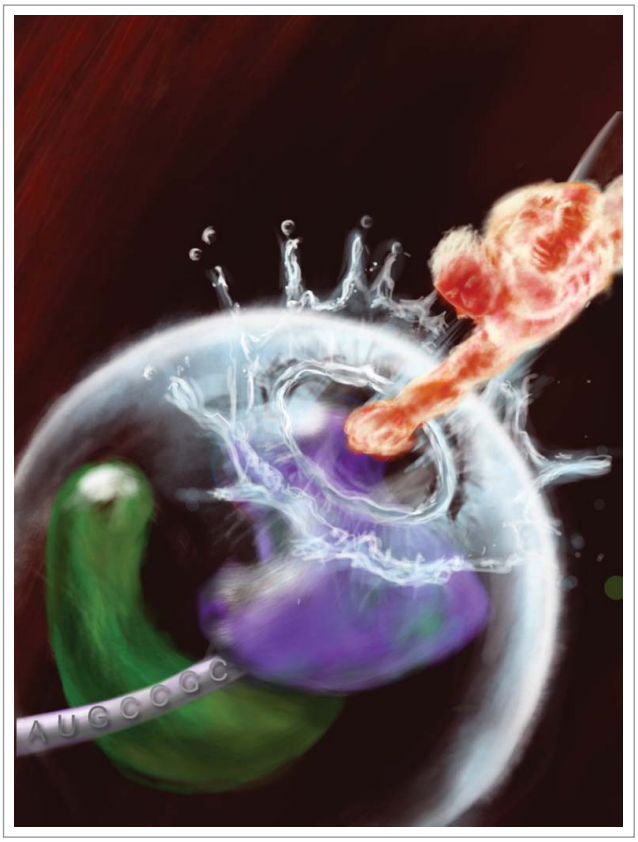

Figure 1. ROS in translation—The battle between oxidants and antioxidants. Protein synthesis is a key physiological task of cancer cells and plays an essential role in oncogenic transformation. Through activation of NRF2, intracellular antioxidants play a critical role in protecting the integrity of the cancer cell translation machinery. As a therapeutic strategy, oxidants can be used to breach the antioxidant protective barrier to hamper protein synthesis and thus pancreatic cancer cell survival.

and mTOR, represent promising targets for cancer therapy. Our study suggests that inhibition of AKT is superior to inhibition of its canonical downstream effector mTOR in the context of PDA, likely due to the inhibitory role of mTOR in the process of macropinocytosis.[5] Although the prototypic mechanism of mTOR regulation in cells is through activation of the PI3K/AKT pathway, an alternative mTOR independent but PI3K dependent pathway regulating translation initiation through 4EBP1 has also been reported.[6] Indeed, mTOR inhibitors exhibit limited efficacy in RAS-mutant cancers in comparison to PI3K/AKT inhibitors, reflecting the complexity of signaling cascades converging upon translational regulation.

A caveat of single agent inhibition of AKT is that it induces negative feedback loops that lead to the activation of receptor tyrosine kinases that mediate resistance.[7] We found that in addition to regulating the activity of the translation machinery, NRF2 also promotes EGF autocrine signaling in KRAS mutant cells, through redox-dependent regulation of ADAM10 activity. Since the integrity of autocrine signaling loops is central to drug resistant mechanisms in pancreatic cancer cells, elevated ROS provided a dual benefit by impairing global mRNA translation and simultaneously blunting drug resistant feedback mechanisms. As such, combined inhibition of AKT signaling and synthesis of glutathione, a vital intracellular antioxidant, synergistically hampered the survival of PDA cells *in vitro* and *in vivo*, presenting a new opportunity for therapeutic intervention.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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