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COMMENTARY

KRas, ROS and the initiation of pancreatic cancer

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ABSTRACT

Oncogenic mutations of *KRAS* are the most frequent driver mutations in pancreatic cancer. Expression of an oncogenic allele of *KRAS* leads to metabolic changes and altered cellular signaling that both can increase the production of intracellular reactive oxygen species (ROS). Increases in ROS have been shown to drive the formation and progression of pancreatic precancerous lesions by upregulating survival and growth factor signaling. A key issue for precancerous and cancer cells is to keep ROS at levels where they are beneficial for tumor development and progression, but below the threshold that leads to induction of senescence or cell death. In KRas-driven neoplasia aberrantly increased ROS levels are therefore balanced by an upregulation of antioxidant genes.

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Epidemiological and animal studies suggest that supplementation of dietary antioxidants decreases cancer risk, which implies that increased ROS may play a role in carcinogenesis.[1] Approximately 95% of all pancreatic ductal adenocarcinoma (PDA) show acquisition of activating *KRAS* mutations,[2] which, due to oncogene-mediated alterations in the cell's metabolism, goes along with increased cellular oxidative stress levels.[3-6] In mouse models for development of PDA, KRas-caused formation of ROS already is induced in acinar cells and gradually increased during ADM and PanIN formation and progression[5] (Fig. 1).

In pancreatic cancer, oncogenic KRas induces the generation of ROS through multiple mechanisms. Typical metabolic changes initiated by tumor cells are, for example, an increase in aerobic glycolysis (Warburg effect) to support growth under hypoxic conditions[7] or altered mitochondrial metabolic activity.[5,6,8-10] Oncogenic KRas can modulate mitochondrial metabolism and ROS generation by regulating hypoxia-inducible factors (HIFs) HIF-1α and HIF-2α,[8] or through regulation of the transferrin receptor (TfR1), which is highly expressed in pancreatic cancers.[11] In addition, KRas can induce suppression of respiratory chain complex I and III to cause mitochondrial dysfunction.[6,12] Decreased mitochondrial efficiency then results in an increased production of ROS.[5] A possible cause is the ROS-mediated occurrence of 4-hydroxy-2-nonenal (4HNE) and 4HNE-adduct formation with macromolecules, which can lead to inhibition of mitochondrial proteins or damage of mtDNA.[5]

KRas-induced increases in intracellular ROS levels can also occur via altered NADPH oxidase activities,[1] i.e. due to activation of Rac1-NOX4 signaling.[13] For example, Rac1 in $Kras^{G12D}$-expressing PanIN1B/PanIN2 is increasingly active when the tumor protein p53-induced nuclear protein 1 (TP53INP1) is knocked out or decreasingly expressed.[14] Other mechanisms by which increases in intracellular ROS can be achieved include enhanced growth factor signaling,[15,16] $KRas^{G12D}$-induced induction of autophagy-specific genes 5 and 7 (ATG5, ATG7),[17] repression of SESN3, which controls the regeneration of peroxiredoxins,[18] or expression of micro RNAs such as miR-155.[19]

In vivo in KC mice the depletion of ROS using NAC or the mitochondrially-targeted antioxidant mitoQ leads to a dramatic decrease in formation and progression of precancerous lesions.[5,14] $KRas^{G12D}$-induced mitochondrial ROS (mROS) engages key-signaling pathways that previously have been linked to development and progression of pancreatic cancer. These include activation of the ERK1/2 signaling pathway,[6] upregulation of epidermal growth factor receptor (EGF-R) signaling,[5] as well as induction of canonical and alternative activation pathways for nuclear factor κ-B (NF-κB),[5] which both have been implicated in the progression of PDA.[20,21]

The serine/threonine kinase Protein Kinase D1 (PKD1) is a major mediator of KRas-mROS signaling,[5,22] however, its activation by mROS most likely is indirect. Previously, it was shown that in response to mROS PKD1 can be activated via Src-mediated phosphorylation

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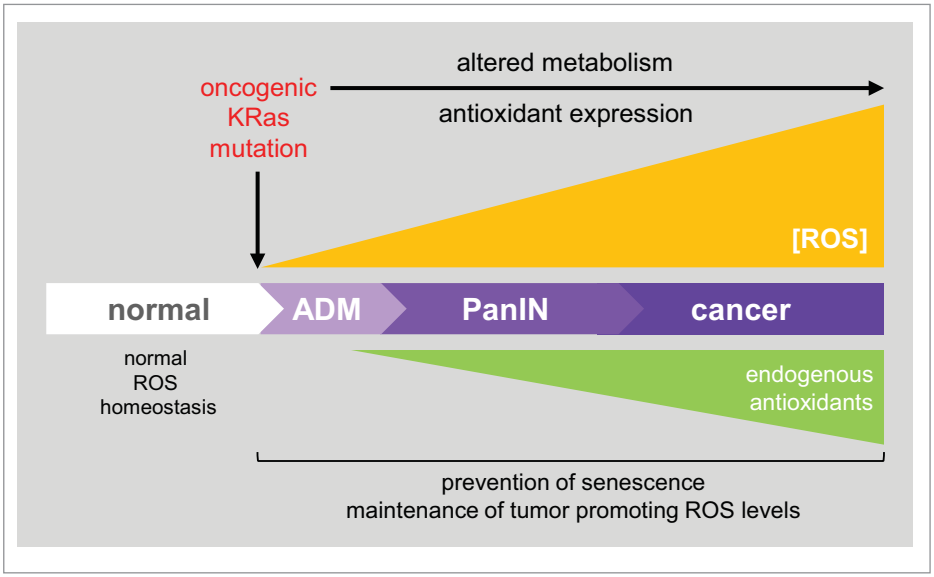

Figure 1. KRas-driven ROS homeostasis and its role in the development of pancreatic cancer. Acquisition of an oncogenic KRas mutation in pancreatic acinar cells leads to their transdifferentiation to duct-like cells. This process named acinar-to-ductal metaplasia (ADM) forms the precursor to PanIN lesions. KRas-induced formation of ROS, due to changes in the cell's metabolic programs, is involved in both ADM and growth and progression of PanIN lesions. A key issue for precancerous and cancer cells is to keep ROS at levels where they are beneficial for tumor development or progression, but below the threshold that leads to induction of senescence or cell death. In KRas-driven neoplasia aberrantly increased ROS levels are therefore accompanied by an upregulation of antioxidant genes.

events.[23-25] Src is a redox-regulated kinase and its activation involves the oxidation of cysteine residues which then results in intramolecular disulfide bond formation and increased kinase activity.[26] This can be further potentiated by ROS-mediated oxidation and inactivation of regulatory phosphotyrosine phosphatases.[27] Although it remains to be tested, arguments for an involvement of Src in the KRas-mROS-PKD1 signaling cascade are recent findings showing cooperation of Src and oncogenic KRas in driving pancreatic neoplasia,[28] metastatic growth and therapy resistance in pancreatic cancer.[29]

PKD1 can activate NF-κB downstream of ROS,[24,25] and during development of PDA, KRas-mROS-PKD1-NF-κB signaling upregulates the expression of EGF-R, its ligands TGFα and EGF as well as their sheddase ADAM17.[5] Overexpression of EGFR and its ligands occurs frequently in the early development process of PDA.[15] It is required to elevate overall KRas activity (oncogenic and wildtype KRas) to pathological levels by additionally activating the wildtype allele.[30-32] An emerging key-role of PKD1 for the initiation of pancreatic cancer is indicated by its additional involvement in the activation of Notch signaling downstream of mutant and wildtype KRas.[22,33] Notch and NF-κB signaling pathways can co-operate to mediate formation of pre-neoplastic lesions.[34] Thus PKD1 brings together 2 important pathways that drive the formation of precancerous lesions.

During development of PDA, the excess of ROS caused by oncogenic KRas needs to be counterbalanced by an increased expression of antioxidant molecules (Fig. 1) in order to generate the pathophysiological conditions under which ROS can mediate cell proliferation[35] and down-regulate tumor suppressors such as INK4/p16 and SMAD4.[9] Otherwise ROS can increase to levels where they induce senescence or cell death.[36] Fine tuning to mitigate the damaging effects of ROS in KRas-driven tumors occurs by upregulation of ROS detoxifying enzymes. This can be mediated via activation of nuclear respiratory factor 2 (Nrf2), a transcription factor that regulates a panel of antioxidant genes. $Kras^{G12D}$ mutations increase the transcription of Nrf2 *in vivo* in KC mice and PDA.[37] Induction of Nrf2 expression is a response to mitochondrial ROS (mROS), since its expression is lost in pancreata of KC mice that were treated with a mitochondrial antioxidant.[5] Acquisition of Nrf2 expression results in low, but pro-tumorigenic levels of ROS in pre-neoplastic pancreatic cells and cancer cells.[14] A similar relationship between ROS and Nrf2 signaling has been described for development and progression of other cancers.[38] Nrf2 activity also can be further increased in pancreatic neoplasia due to somatic mutations that disrupt the interaction with its inhibitor Keap1.[39]

Besides Nrf2, Pim kinases also contribute to $KRas^{G12V}$-driven modulation of cellular ROS levels by regulating levels of glutathione peroxidase 4 and peroxiredoxin 3 or expression of manganese superoxide dismutase (MnSOD), which is encoded by the SOD2 gene.[40]

If Pims are absent, c-Myc can compensate by regulating expression of the SOD2 gene.[40] Similarly, upregulation of SOD2 expression is mediated in response to mROS via PKD1-NF-κB signaling.[24] The product of MnSOD activity is an increase in hydrogen peroxide, a *bona fide* signaling molecule that is important for tumor cell proliferation.[16] It should be noted that besides upregulation of antioxidant systems, in pancreatic cancer, KRas activates multiple other mechanisms that contribute to prevent cell death and senescence. These include upregulation of the transcription factor Twist[41] and signaling that bypasses retinoblastoma (Rb) protein.[42]

In summary, during the development and progression of PDA, oncogenic KRas causes metabolic changes that lead to increased generation of mitochondrial reactive oxygen species. Oncogenic KRas also upregulates antioxidant systems to balance ROS to levels at which they drive major signaling pathways that contribute to oncogenic transformation and tumor progression.[5,30,32] A key question now is if the knowledge can be applied for developing novel therapeutic approaches. One possibility is to inhibit expression or activity of Nrf2, with the overall goal to reduce induction of antioxidant systems and to drive KRas-generated ROS to levels where they induce senescence or cell death. Such an approach may be most effective in combination with chemotherapeutic drugs that additionally increase cellular ROS production. Another possibility is to administer mitochondrially-targeted antioxidants such as mitoQ, with the overall goal to suppress KRas-induced mROS formation to target development and progression of PDA. While approaches aiming on increasing ROS levels may be more efficient in targeting progressed PDA, approaches aiming on decreasing ROS levels may be more efficient as a cancer prevention strategy.

Abbreviations

ADM	acinar-to-ductal metaplasia
EGF	epidermal growth factor
EGF-R	epidermal growth factor receptor
KC	mice expressing oncogenic KRas under a pancreatic acinar cell-specific promoter
KRas	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
Kras	mouse gene encoding KRas
KRAS	human gene encoding KRas
mROS	mitochondrial reactive oxygen species
NAC	N-acetyl-L-cysteine
NF-κB	nuclear factor κ-B
Nrf2	nuclear respiratory factor 2
PanIN	pancreatic intraepithelial neoplasia
PDA	pancreatic ductal adenocarcinoma
PKD1	protein kinase D1
ROS	reactive oxygen species
TGFα	transforming growth factor-α.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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