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KRAS-driven ROS promote malignant transformation

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The mechanism underlying KRAS (Kirsten rat sarcoma viral oncogene homolog)-driven cellular transformation remains unclear because of the complexity of its downstream effectors. Park et al. recently reported that levels of reactive oxygen species (ROS) are increased by KRAS and are responsible for KRAS-driven malignant transformation, and further identified the signaling cascade involved as KRAS/p38/PDPK1/PKCδ/p47^{phox}/NOX1. These findings provide new insight into the molecular mechanisms governing KRAS-driven malignant transformation.

The discovery that KRAS (Kirsten rat sarcoma viral oncogene homolog) alone can transform normal fibroblasts into malignant neoplasms was expected to contribute to our understanding of cancer pathogenesis. However, the molecular mechanism underlying KRAS-driven cell transformation remains obscure because it is complicated by the large cohort of downstream effectors of KRAS. In this context, many studies have suggested that reactive oxygen species (ROS) production is responsible for KRAS-induced cellular transformation.

Classically, ROS have been simply viewed as the unregulated by-products of aerobic metabolism and other enzymatic processes. However, ROS are not only a harmful by-product of the respiratory chain in mammalian cells but also serve as signaling molecules, participating in a variety of cellular signaling pathways such as growth factor pathways,[1] inflammation,[2] engagement of integrins,[3] and adhesion to the extracellular matrix.[4]

ROS acting as signaling molecules are purposely generated through nicotinamide adenine dinucleotide phosphate (NADPH) oxidases that are plasma membrane-bound enzyme complexes, rather than in mitochondria. Since ROS display both beneficial effects as signaling molecules and harmful effects as oxidative by-products, the pathways that regulate ROS homeostasis may be crucial for cellular function. Disturbance of ROS homeostasis can lead to damage of important components of the cell, including proteins, lipids, and DNA, with potential impact to the whole organism and an increased risk of mutagenesis. Indeed, an aberrant increase in intracellular ROS has been implicated in a large number of diseases including cancer.[5] Previous studies have suggested that many types of cancer cell have increased levels of ROS.[6] In this regard, Park et al. reported that intracellular ROS levels can be increased by KRAS signaling and are responsible for KRAS-induced cellular transformation. The *KRAS* gene is frequently mutated and KRAS protein aberrantly activated in many types of cancer, including pancreatic, thyroid, colon, lung, and liver cancers, and in myelodyspastic syndrome, and such mutations are correlated with poor prognosis.[7] Importantly, Park et al. dissected a novel signaling axis governing KRAS-induced ROS production during KRAS-driven cellular transformation[8] (**Fig. 1**). They clearly showed that transient expression of KRAS promotes ROS production in normal fibroblasts together with cellular transformation. Moreover, ectopic expression of antioxidant enzymes abolishes KRAS-driven cellular transformation, indicating that KRAS transforms normal fibroblasts through ROS production. Importantly, they found that KRAS induces ROS production through activation of NADPH oxidase 1 (NOX1) among the NOX family. In further analysis, NOX1 was found to be activated by a cytosolic regulatory subunit p47^{phox} (also known as neutrophil cytosolic factor 1 or

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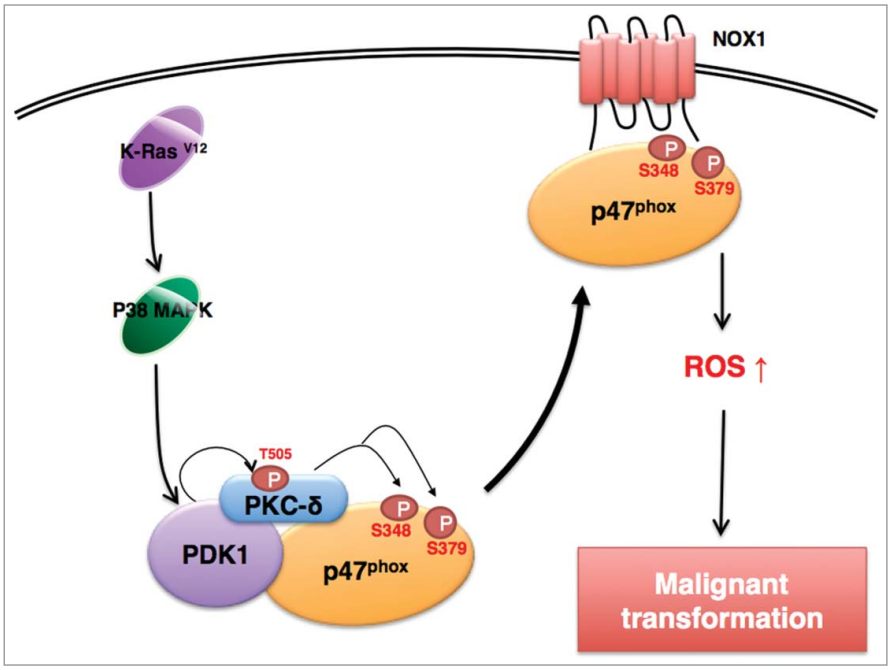

Figure 1. Schematic model for KRAS-driven signals leading to ROS production and consequent malignant transformation. Note that PKCδ, PDPK1, and p47phox form a protein complex, thereby facilitating the signal cascade for ROS production. KRAS, Kirsten rat sarcoma viral oncogene homolog; PKC, protein kinase C; PDPK1, 3-phosphoinositide-dependent protein kinase-1; ROS, reactive oxygen species.

NCF1). By a mechanistic approach, the authors further demonstrated that, among the protein kinase C (PKC) isoforms, PKCδ (also known as PRKCD) is activated by 3-phosphoinositide-dependent protein kinase-1 (PDPK1) in a KRAS-dependent manner and sequentially activates $p47^{phox}$ by phosphorylation on Ser348 and Ser379, thereby causing translocation of $p47^{phox}$ into the membrane fraction for NOX1-catalyzed ROS production. Of note, PDPK1 is phosphorylated on Thr505 by p38 (also known as mitogen-activated protein kinase 14 or MAPK14) in a KRAS-dependent manner and then activates PKCδ, which consequently binds to the SH3-N domain of $p47^{phox}$ and catalyzes phosphorylation on Ser348 and Ser379 residues of the $p47^{phox}$ C-terminal region. It is possible that direct binding of PKCδ to the SH3-N domain in $p47^{phox}$ increases the proximity and orientation of the active site in PKCδ, facilitating phosphorylation of Ser348 and Ser379 in $p47^{phox}$. Moreover, Park et al. showed that PKCδ, PDPK1, and $p47^{phox}$ proteins form a signal transduction complex that facilitates KRAS-induced ROS production.

Numerous studies have clearly shown that KRAS induces activation of ERK (also known as mitogen-activated protein kinase 3 or MAPK3), JNK (also known as mitogen-activated protein kinase 8 or MAPK8), and p38 MAPK signaling.[9,10] In line with these previous studies, Park et al. found that p38 is involved in KRAS-induced ROS production and malignant transformation. Inhibition of p38 blocked KRAS-induced ROS production, anchorage-independent colony formation, and *in vivo* tumor formation. In parallel, overexpression of p38 increased ROS production even in the absence of $KRAS^{V12}$. Although inhibition of ERK also blocked anchorage-independent colony formation, it did not affect ROS production in $KRAS^{V12}$-expressing cells, indicating that ERK might be involved in a ROS-independent pathway for KRAS-induced malignant transformation. Extending their observation, Park et al. also found that endogenous oncogenic KRAS in cancer cells induces ROS production through the same signaling cascade as exogenous mutant $KRAS^{V12}$ in normal fibroblasts.

For cancer prevention and treatment, it is a prerequisite to understand the molecular mechanisms underlying cancer initiation and progression. For this reason, identification of the molecular mechanisms underlying KRAS-induced ROS production during cellular transformation might contribute to our understanding of cancer pathogenesis and the development of novel therapeutic agents for cancer. In this regard, Park et al. demonstrated that $KRAS^{V12}$ induces ROS production via the signaling axis p38/PDPK1/PKCδ/$p47^{phox}$/NOX1 in cancer cells as well as normal fibroblasts (**Fig. 1**). Furthermore, inhibition of single components of this signaling axis effectively abolished KRAS-driven ROS production and malignant transformation. These findings may provide new insight into the molecular mechanisms governing KRAS-driven cellular transformation and cancer progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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