KRAS-induced Actin-interacting Protein: A Potent Target for Obesity, Diabetes and Cancer

TAKAHIRO FUJIMOTO and SENJI SHIRASAWA

Department of Cell Biology, Faculty of Medicine and Central Research Institute for Advanced Molecular Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Abstract. KRAS-induced actin-interacting protein (KRAP) was originally identified as one of the genes deregulated in colorectal cancer. KRAP encodes a cytoplasmic protein associated with filamentous-actin (F-actin), and the amino acid sequences are highly conserved among KRAP orthologues from fish to mammalian species. We demonstrated that KRAP-deficient mice show altered whole-body energy metabolism and resistance to diet-induced obesity and diabetes. Although the precise mechanisms underlying the metabolic phenotypes in the KRAP-deficient mice remain unclear, KRAP is considered to be a target for metabolism-related diseases. Furthermore, several groups have reported that KRAP is a cancer-associated gene. Further studies on the molecular functions of KRAP in physiological tissues could provide a better understanding of various diseases, and opportunities for intervention in various human diseases. In this review, we summarize the current understanding of KRAP and the roles that it plays in a variety of diseases.

Identification and Characterization of KRAP

The RAS GTPases transmit signals from tyrosine kinases at the plasma membrane to a variety of serine/threonine kinases, which deliver signals to the nucleus. RAS proteins and their relatives play critical roles in diverse cellular processes, including proliferation, differentiation, apoptosis, cytoskeletal organization, cell polarity, movement, and gene expression (1, 2). Mutations in the KRAS gene are frequently observed in colorectal, pancreatic and lung cancer (3-5). These mutations are invariably point mutations that render the proteins constitutively active (4). However, KRAS-targeted therapy has not yet been clinically developed, and patients with colorectal cancer bearing mutated KRAS did not benefit from cetuximab, a monoclonal antibody against the epidermal growth factor receptor (5). Therefore, elucidating the precise molecular mechanisms of activated KRAS in the development and malignancy of human cancer will be needed to determine whether it can represent a target for cancer therapy.

As a model system for elucidating the functions of activated KRAS in colorectal cancer, we established HKe3 cells, in which the mutated (G13D) KRAS allele was deleted from HCT116 cells by homologous recombination (6). By using this model system, we and colleagues reported several pieces of evidence demonstrating the importance of the functions of activated KRAS in cancer development and progression (6-10). Furthermore, among the various genes whose expression levels are deregulated by mutated KRAS in the model system, epiregulin (EREG), a member of the epidermal growth factor family, was found to play a critical role not only in tumorigenesis, but also in dermatitis and immune-related responses (8, 11, 12), suggesting that KRAS-regulated genes may also take part in important biological processes besides tumorigenesis. Another molecule, KRAS-induced actin-interacting protein (KRAP) was deregulated in the colorectal cancer HKe3 cells, and was found to have a role in metabolism (13).

Identification and Characterization of KRAP

The human KRAP gene was originally identified as one of the genes whose expression level was up-regulated by activated KRAS in human colon cancer HCT116 cells (14). While KRAP is minimally expressed in the normal colon epithelium, deregulated constitutive KRAP expression is observed in colon cancer cells, including the DLD-1, WiDr, Colo201, LoVo and SW620 colon cancer cell lines (14). The KRAP amino acid sequence is well conserved from fish to mammalian species, although minor changes have occurred during evolution (14) (Figure 1). Mouse KRAP has high amino acid sequence identity (80% homology) to human KRAP. In mouse tissues,
the KRAP protein is widely detected, with high levels in the pancreas, liver, brown adipose, testis, kidneys and brain, and with low levels in lungs, spleen, small intestine, heart and skeletal muscle (15). However, of note is that KRAP expression is restricted to a particular cell type in each organ, i.e. KRAP is predominantly expressed in hepatocytes but not in the other constituent cells, such as Kupffer cells, Ito cells, ductal cells or endothelial cells in the liver (15). In the pancreas, KRAP is predominantly expressed in the exocrine acinar cells, not in the endocrine islet cells (15). Although information about the distribution of KRAP in other organs awaits future studies, KRAP is also expressed in a specific cell type within other organs as well.

Previous studies have identified the subcellular distribution of KRAP in mouse tissues under physiological conditions and in human cancer cells (14, 15). Remarkably, KRAP localization is restricted to the apical pole of hepatocytes and pancreatic acinar cells. Even though it remains unclear whether KRAP is localized within microvilli, cell−cell junctions such as adherence junctions and tight junctions, and/or other structural apparatus, KRAP appears to associate with F-actin (14, 15). These observations lead us to expect that the coiled-coil region predicted by the amino acid sequence around the carboxyl-terminus of KRAP may be responsible for the association between KRAP and F-actin. Indeed, a deletion mutant KRAP protein which lacks the carboxyl-terminal region containing the

Figure 1. A phylogenetic tree for KRAP constructed from multiple alignments using the CLUSTALW program. The horizontal branch lengths are proportional to the rates of phylogenetic change.

Figure 2. The carboxyl-terminal domain containing the coiled-coil region of KRAP is responsible for its localization and interaction with the F-actin stress fibers in NIH3T3 cells. A: A schematic illustration of mouse KRAP (top) and KRAP-deletion mutants used in this study (bottom). B: Confocal images of NIH3T3 cells transfected with HA-tagged KRAP deletion mutants with anti-HA and phalloidin. Four independent experiments were performed, and the localization of KRAP was analyzed against 100 transfectants in each experiment. The summarized data are presented as the means±s.e.m. in (A). a.a., Amino acids; N, amino-terminus; C, carboxyl-terminus; scale bar 50 μm.

Figure 3. KRAP-deficient mice are resistant to obesity. A: A photograph of KRAP-deficient (KO) and the wild-type (WT) littermate mice after 13 weeks on a high-fat diet. B: A histological analysis of the epididymal white adipose tissue of WT and KO mice after 13 weeks of a high-fat diet by hematoxylin and eosin staining. Scale bar 100 μm.
coiled-coil region (deltaC) appeared not to localize along F-actin stress fibers in NIH3T3 cells (Figure 2), whereas other deletion mutants, deltaCC, CC+ and CC−, as well as the full-length KRAP protein, appeared to localize with F-actin in these cells (Figure 2). Thus, not only the predicted coiled-coil region, but also the region adjacent to the coiled-coil region of the carboxyl-terminus of KRAP may be crucial for its interaction with the cytoskeleton or for directional targeting of the KRAP protein toward the apical pole in polarized epithelial cells such as hepatocytes and pancreatic acinar cells. In contrast to the restricted subcellular localization of KRAP in normal tissues, KRAP is distributed throughout the cytoplasm and is present in both the apical and basolateral regions in cancer cells, although the association of KRAP with F-actin is still observed (14). Taken together, these results may suggest the existence of distinct KRAP functions according to its subcellular localization or cellular status, hence the functional aspects of KRAP in both the normal polarized epithelium and deregulated malignant cells should be assessed to better understand the significance of KRAP in cancer development and progression.

**KRAP as a Target for Obesity and Diabetes**

KRAP-deficient (knockout, KO) mice were established to clarify the physiological significance of KRAP in vivo (13). KRAP-KO mice display a profound metabolic phenotype that includes an increased metabolic rate, reduced adiposity, improved glucose tolerance, hypoinsulinemia and hypoleptinemia. More importantly, KRAP-KO mice are protected against diet-induced weight gain, fatty liver formation and insulin resistance under a high-fat diet, implicating KRAP as a potential target for the prevention or treatment of obesity and related diseases.

The KRAP-KO offspring appear normal, and their total body weight at birth is also normal. KRAP-KO mice are fertile and have a normal life span. However, KRAP-KO mice show reduced weight gain and a lean appearance after weaning relative to their wild-type littermates under both normal and high-fat diets (Figure 3), despite the fact that the KRAP-KO mice consume sufficient food (13). The reason underlying the lean phenotype of the KRAP-KO mouse is therefore of great interest. The first clue to this effect is that the KRAP-KO mice have an enhanced metabolic rate, both in the daytime and at night. The possibility that KRAP-KO mice have a lean phenotype due to the results of poor nutrient absorption is not likely, because the quantitative and qualitative assessments of feces and a food tolerance test indicate that these parameters in KRAP-KO mice are normal. Taken together, these data show that KRAP-KO mice have increased energy turnover, which leads to their resistance to diet-induced obesity.
Obese individuals have an increased risk of insulin resistance, progressing to type 2 diabetes (16-18). To determine whether KRAP might also regulate this process, glucose metabolism in the KRAP-KO mice was examined, and it was observed that KRAP-KO mice display improved glucose tolerance with lower insulin levels compared with wild-type mice (13). More importantly, KRAP-KO mice are protected against high-fat diet-induced insulin resistance and glucose intolerance. With regard to the mechanism(s) underlying these effects, it has been demonstrated that KRAP does not directly affect insulin signaling (13). In contrast, insulin-independent glucose uptake in brown adipose tissues (BAT) of KRAP-KO mice is enhanced, suggesting that the deregulated BAT functions, at least in part, play important roles in the improved glucose tolerance and altered energy homeostasis in the mice. Considering the recent findings that adult humans have functional BAT (19-22), the activation of BAT upon KRAP suppression might be a novel therapeutic approach to combat obesity and diabetes.

KRAP-KO mice also have a decreased triglyceride content in their white adipose, BAT and liver tissues (13). Consistent with the reduced triglyceride content, the expression levels of lipogenic genes encoding acetyl-CoA carboxylase (ACC)-2, ACC-1, fatty acid synthase, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) are decreased in the livers of KRAP-KO mice (13). Among these factors, ACC-2 is a critical regulator of the balance between the synthesis and oxidation of fatty acids (23). ACC-2-deficient mice and stearoyl-CoA desaturase-1 (SCD1)-deficient mice have a metabolic phenotype associated with an increased metabolic rate and decreased adiposity (24-26), which is similar to that observed in the KRAP-KO mice. Additionally, HMG-CoA reductase is the rate-limiting enzyme for cholesterol biosynthesis. Taken together, these findings suggest that the decrease in expression of fatty acid synthesis-related genes, including ACC2, SCD1 and HMG-CoA reductase, in the liver of KRAP-KO mice might function as a critical mediator of the altered energy homeostasis in KRAP-KO mice. Besides the liver, BAT also showed a decreased expression of fatty acid desaturases, including SCD1, in KRAP-KO mice (13), which might also account for the different metabolic phenotypes in the mice.

Although the KRAP-KO mice demonstrate a profound metabolic phenotype including an enhanced metabolic rate, improved insulin-independent glucose tolerance and decreased adiposity, as described above, the precise mechanism(s) underlying the phenotype caused by KRAP deletion remain unclear due to the lack of conditional knockout of KRAP (13). Furthermore, the cell-specific effects of KRAP deletion on energy, glucose and lipid metabolisms remain ambiguous, however, improved glucose tolerance and decreased adiposity in the BAT of KRAP-KO mice is not likely due to the cell-specific effects of KRAP deletion (13). It is therefore more likely that certain inputs from other organs to the BAT would be deregulated in the KRAP-KO mice. Considering a recent report indicating that identification of networks, rather than one or two genes, is important in understanding the molecular pathology of diseases (27, 28), clarification of KRAP functions, not only in each tissue, but also in systemic inter-tissue communication in which multiple tissues participate, is necessary to validate KRAP as a drug target for obesity and diabetes (Figure 4).

**KRAP as a Potential Target for Cancer**

Human KRAP was originally identified as one of the deregulated genes in colorectal cancer (14). Furthermore, growing evidence supporting KRAP as being a cancer-associated gene has emerged as follows. KRAP is associated with liver-preferential metastasis of small cell lung cancer (29); KRAP expression is highly associated with lung squamous cell cancer, but not with normal cells, breast cancer cells, renal cell cancer or lung adenocarcinoma (30); KRAP is down-regulated by p38 MAPK inhibition in transformed follicular lymphoma (31); KRAP appears to affect prostate cancer progression into an androgen-independent state (32); and KRAP expression is associated with the proliferative phenotype in chronic lymphocytic leukemia (33). Taken together, these data suggest that KRAP expression is altered upon the development of malignancy and/or by the extracellular microenvironment, and that understanding the regulatory mechanisms and the significance of the changes in the expression of KRAP are important in determining cancer phenotypes. Recently, we identified a signaling molecule as a KRAP-binding protein and obtained evidence for the linkage between KRAP and the signaling molecule in both cancer cells and physiological tissues. Thus, more detailed functional studies of the role of KRAP in cancer could provide a novel avenue leading to new cancer therapeutic strategies in the future.

**References**
