Oncogenic KRAS triggers metabolic reprogramming in pancreatic ductal adenocarcinoma

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an extremely high lethality rate. Oncogenic KRAS activation has been proven to be a key driver of PDAC initiation and progression. There is increasing evidence that PDAC cells undergo extensive metabolic reprogramming to adapt to their extreme energy and biomass demands. Cell-intrinsic factors, such as *KRAS* mutations, are able to trigger metabolic rewriting. Here, we update recent advances in KRAS-driven metabolic reprogramming and the associated metabolic therapeutic potential in PDAC.

Key words: pancreatic ductal adenocarcinoma, oncogenic KRAS activation, metabolic reprogramming

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers, with a 5-year survival rate of 9% in the USA, and is projected to become the second-leading cause of cancer-related death in the near future.[1] Advancements in fundamental and adjuvant chemotherapy have been made in PDAC patients, but only modest incremental progress in patient outcomes has been made. PDAC is a disorder with multiple genetic mutations during multistage progression.[2] The most important genetic event in the development of PDAC is an oncogenic mutation of *KRAS* (90% in TCGA-PAAD). KRAS is a member of the Rat sarcoma (RAS) family of guanosine triphosphate (GTP)-ases whose activity is regulated by the guanosine diphosphate (GDP)/GTP cycle and is involved in several cellular processes, including survival, proliferation, differentiation, migration, and apoptosis.[3] In contrast to the tight regulation of wild-type protein, mutant KRAS leads to the persistent activation of downstream signaling pathways, such as Raf/MEK/ERK, PI3K/PTEN/AKT, and Ral guanine nucleotide exchange factor (Ral-GEF).[4] Using a mouse disease model, oncogenic *KRAS* mutations have been proven to initiate acinar-to-ductal metaplasia (ADM) and promote and maintain pancreatic intraepithelial neoplasia (PanIN) lesions.[5] Mutations of *KRAS* alone do not recapitulate the full spectrum of PDAC development. In addition, the loss of tumor suppressor genes (*e.g.*, tumor protein p53 [*TP53*]), epigenetic dysregulation (*e.g.*, lysine-specific demethylase 6A [*KDM6A*], SET Domain Containing 2 [*SETD2*]), and/or environmental stresses are essential for PDAC malignant transformation and progression.[6]

Tumors are usually accompanied by unique metabolic disorders, which can be regarded as "metabolic diseases", and many of studies have confirmed that targeting key metabolic pathways can indeed suppress tumor growth.[7] To continuously fulfill biosynthetic demands, tumor cells usually reprogram the glucose metabolism process, which gives priority to glycolysis (the Warburg effect) for energy supply even in an environment with sufficient oxygen.[8] This process is characterized by increased glucose consumption, decreased oxidative

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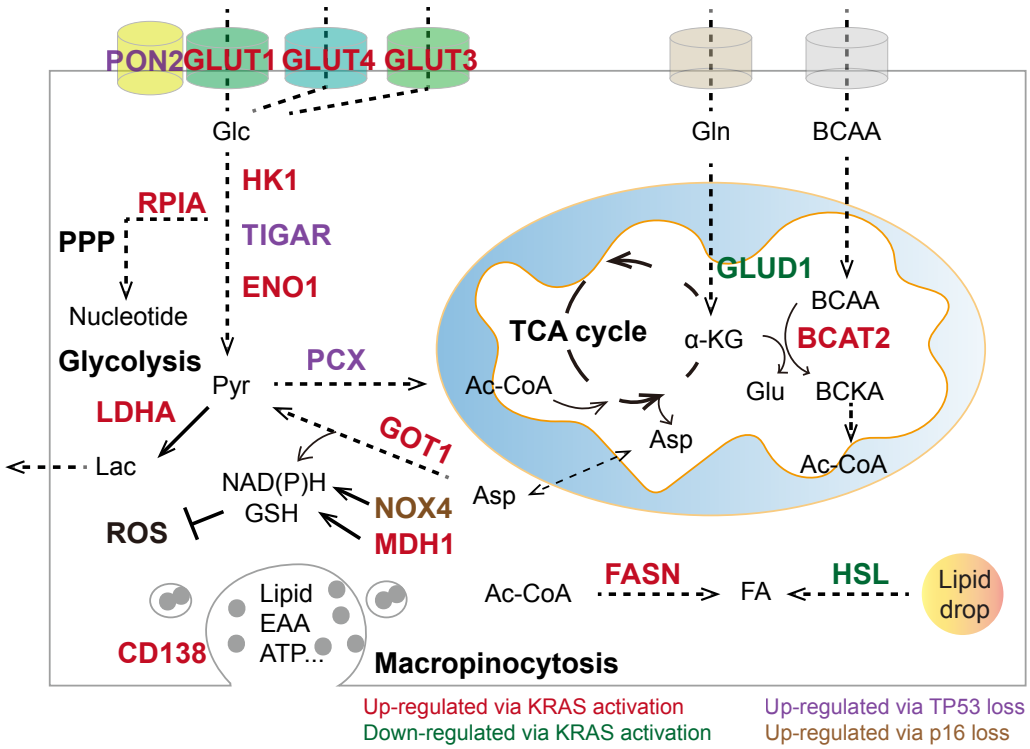

Figure 1: Summary of metabolic pathways and enzymes influenced by *KRAS*, TP53, and p16. PON2: paraoxonase 2; GLUT1: glucose transporter 1; GLUT3: glucose transporter 3; GLUT4: glucose transporter 4; HK1: hexokinase 1; TIGAR: Trp53-induced glycolysis regulatory phosphatase; ENO1: alpha-enolase; LDHA: lactic dehydrogenase A; RPIA: ribose 5-phosphate isomerase A; Pcx: pyruvate carboxylase function; BCKA: branched-chain α-keto acids; BCAT: branched-chain aminotransferases; GLUD1: glutamate dehydrogenase 1; GOT1: aspartate transaminase; NOX4: NAD(P)H oxidase 4; MDH1: malate dehydrogenase 1; HSL: hormone-sensitive lipase; FASN: fatty acid synthase; Glc: glucose; Lac: lactate; Pyr: pyruvate; Ac-CoA: acetyl-CoA; αKG: α-ketoglutarate; Gln: glutamine; Glu: glutamate; Asp: aspartate; BCAA: branched-chain amino acids; FA: fatty acids; EAA: essential amino acids; NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; GSH: glutathione; PPP: pentose phosphate pathway; TCA cycle: tricarboxylic acid cycle; ROS: reactive oxygen species.

phosphorylation, and enhanced lactate synthesis.[9] Lactate in turn promotes angiogenesis and immune cell trafficking in tumors.[10] In addition, the accumulation of reactive oxygen species in proliferating tumor cells leads to DNA damage, and elevated levels of the pentose phosphate pathway (PPP) generate more nucleotides and reduce nicotinamide adenine dinucleotide phosphate (NADPH) for DNA repair and oxidation resistance.[11] Other metabolites, such as amino acids, fatty acids, and ketone bodies, also participate in tumor metabolism. Moreover, tumor cells can develop autophagy that provides energy in the form of glucose, lactate, amino acids, free fatty acids, and nucleosides for tumor progression, eventually leading to tumor cachexia.[12, 13]

Several studies have shown that tumor cells carry hallmarks of sustained proliferation, enabling replicative immortality, evading growth suppressors, and resisting cell death.[14] To support these extraordinary energetic and biosynthetic demands, tumorigenesis usually accompanies metabolic reprogramming.[14] Early reports have suggested that most tumor cells undergo a metabolic shift toward glycolysis (Warburg effect) to produce energy and toward anabolic pathways to synthesize proteins and lipids, while normal cells mainly depend on oxidative phosphorylation (OXPHOS) in the mitochondria.[15, 16] However, recent reports have begun to uncover the essential role of OXPHOS in tumor cells.[17] Defining how genetic mutations reprogram cellular metabolism has also aroused great interest, which helps us to better understand the intertumoral metabolic heterogeneity and may develop potential targets for cancer therapy. Notably, *KRAS* mutation-driven metabolic reprogramming in PDAC is the most studied. Here, we summarize the latest studies exploring how *KRAS* mutation reprograms metabolic processes to enforce PDAC tumorigenesis (Figure 1).

GLUCOSE METABOLISM

Oncogenic KRAS activation is closely related to tumor-associated glucose metabolic dysfunction.[18] Accumulating evidence has revealed that murine Kras activation enhances glucose uptake and glycolysis by upregulating the transcriptional level of glucose transporters, including

Slc2a1 (encoding Glut1) and *Slc2a4* (encoding Glut4), as well as rate-limiting enzymes, including *Hk1*, *Eno1* and *Ldha*.[19-22] Coherently, the enhanced glycolytic flux caused by oncogenic KRAS can maintain PDAC progression by diverting into anabolic pathways, including the hexosamine biosynthesis pathway (HBP) and nonoxidative PPP, for NADPH production, reactive oxygen species (ROS) detoxification, and nucleotide precursor ribose 5-phosphate synthesis.[20, 23-25] Mechanistically, sustained KRAS can activate the mitogen-activated protein kinase (MAPK) pathway and MYC proto-oncogene (MYC) and hypoxia inducible factor 1 subunit alpha (HIF1α) to transcriptionally regulate transporters and enzymes in glucose metabolism.[20] These metabolic alterations triggered by oncogenic KRAS may confer distinct survival advantages to PDAC under unfavorable microenvironmental conditions.

Moreover, loss of tumor suppressor genes, such as *TP53* and cyclin-dependent kinase inhibitor 2A (*CDKN2A* or *p16*), coordinates with oncogenic *KRAS* mutations to modulate the glycolytic pathway.[19] In a mouse PDAC model, loss of *Tp53* further enhances glycolytic flux and energy supply in multiple ways, including elimination of the transcriptional arrest of glucose transporters (*e.g.*, *Slc2a1* and *Slc2a4*) and rate-limiting enzymes (*e.g.*, *Pgm*, phosphoglycerate mutase) and inhibition of the glycolysis inhibitor *Tigar* (TP53-induced glycolysis and apoptosis regulator).[26-30] Loss of *Tp53* also transcriptionally increases paraoxonase 2 (*Pon2*), which facilitates pancreatic cancer growth and metastasis by stimulating Glut1-mediated glucose transport.[19] Notably, Lowe's group found that restoration of p53 function in *Kras* and *Tp53* mutant-derived PDAC could rewire glucose and glutamine metabolism to favor the accumulation of αKG at the expense of succinate, which triggered chromatin modification 5-hydroxymethylcytosine (5hmC) to facilitate tumor differentiation and blunt tumor cell fitness.[31] Upon oncogenic activation, further loss of p53 prevents these metabolic effects and enables tumor cells to transition to more aggressive and less differentiated PDAC. Moreover, oncogenic KRAS activation in conjunction with inactivated CDKN2A upregulates the expression of NAD(P)H oxidase 4 (*NOX4*) to generate nicotinamide adenine dinucleotide (NAD)+ and supports glycolysis in human and mouse PDAC cell lines.[32]

AMINO ACIDS

Recent studies have suggested that the involvement of amino acids in cancer metabolism is more important than previously thought.[33, 34] The nonessential amino acid glutamine is a common source of carbon and nitrogen for tumor cells.[35, 36] Oncogenic KRAS acts as a converter of glutamine metabolism in PDAC by shifting glutamine metabolism from the TCA cycle to the noncanonical pathway.[37-39] KRAS activation in human PDAC cells downregulates the glutamate dehydrogenase (GLUD1)-dependent canonical Gln utilization pathway but upregulates aspartate transaminase (GOT1) to maintain redox balance, which contributes to cell proliferation and tumor progression.[40, 41] In addition, KRAS can also preserve glutamine metabolism by protecting MDH1 from CARM1-mediated methylation, which indicates its inactive state.[42] In addition, oncogenic KRAS is able to upregulate the mRNA level of nuclear factor-like 2 (*NRF2*), which further reprograms glucose and glutamine into anabolic and antioxidant pathways.[38, 43, 44]

In addition to glutamine, tumor progression still relies on the essential branched-chain amino acids (BCAAs), which refer to leucine, isoleucine, and valine. BCAAs can be converted to glutamate and branched-chain α-keto acids (BCKAs) in the cytosol and mitochondria, respectively, by branched-chain aminotransferases (BCATs), to produce energy and nitrogen for biosynthesis.[13, 45] A recent study showed that KRAS stabilized BCAT2 instead of BCAT1 via spleen tyrosine kinase (SYK) and E3 ligase tripartite-motif-containing protein 21 (TRIM21).[46, 47] BCAT2 is markedly elevated in mouse models and human PDAC, and specific deletion of *Bcat2* in the murine pancreas largely impedes the early stage of PDAC development. Functionally, BCAT2 enhances BCAA uptake to sustain BCAA catabolism and mitochondrial respiration.[48]

FATTY ACIDS AND LIPIDS

In PDAC, obesity and excess fatty acids accelerate tumor growth and metastasis, and lipolysis and lipogenesis processes are indispensable for tumor growth and invasion.[49-52] During pancreatic cancer progression, several catalyzed enzymes related to *de novo* fatty acid and cholesterol synthesis are significantly upregulated, including citrate synthase (*CS*), ATP citrate lyase (*ACLY*), fatty acid synthase (*FASN*), and 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGCR*).[53, 54] FASN, the key enzyme that converts sugar metabolism to fatty acids and palmitate, is highly expressed in both human PDAC tissues and spontaneous mouse models and is associated with poor prognosis in PDAC patients.[55] KRAS sensitizes epidermal growth factor receptor (EGFR) signaling and upregulates *FASN* expression during PDAC progression.[56, 57] Oncogenic KRAS activation in human PDAC cells also enhances lipid droplet accumulation and attenuates fatty acid oxidation by restraining hormone-sensitive lipase (*HSL*) levels.[58] Thus, the stored lipid drop will be utilized as an energy supply to promote the invasion process.[58] These findings have revealed novel mechanisms by which KRAS regulates lipid metabolism to favor PDAC metastasis and invasion.[58, 59]

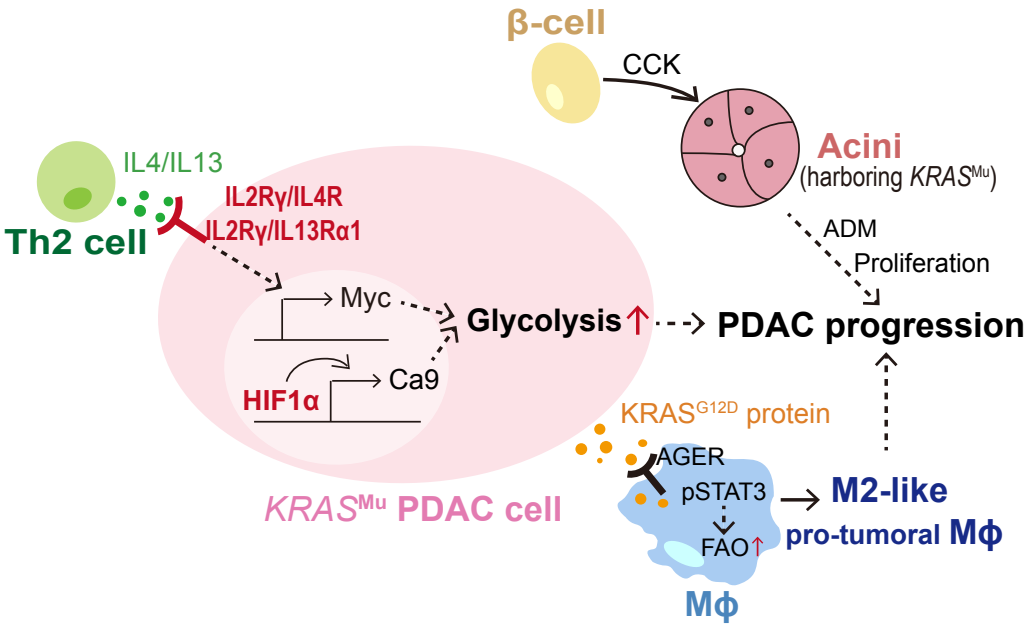

Figure 2: Summary of tumor microenvironment driven by oncogenic KRAS. PDAC: pancreatic ductal adenocarcinoma; FAO: fatty acid; Mϕ: macrophage; ADM: acinar-to-ductal metaplasia; CCK: cholecystokinin; HIF1α: hypoxia inducible factor 1 subunit alpha; CA9: carbonic anhydrases 9; IL4/IL13: interleukin 4/interleukin 13.

NUCLEOTIDE SYNTHESIS

In addition to ribose biogenesis influenced by glucose metabolism, oncogenic KRAS can support PDAC proliferation by activating the MAPK-dependent MYC-RPIA axis.[20] Ribose 5-phosphate isomerase A (RPIA) catalyzes the conversion between ribulose-5-phosphate and ribose-5-phosphate in a nonoxidative PPP pathway, which is the dominant mechanism of nucleotide synthesis for PDAC cells.[60]

MACROPINOCYTOSIS

In addition to the metabolic pathway of single metabolites, tumor cells often develop micropinocytosis, macropinocytosis, and autophagy processes that obtain essential nutrients by engulfing and digesting extracellular matrix or other cells to support their rapid division or proliferation.[18, 61-63] Autophagy is employed to degrade intracellular components and provide energy, ATP, and metabolites, including amino acids, lipids, sugars, and nucleosides, to promote and/or inhibit tumor progression.[64, 65] In fact, a wide range of studies have shown that enhanced macropinocytosis and subsequent hydrolysis of extracellular proteins in lysosomes become significant features of tumoral KRAS activation in both mouse models and primary human PDAC specimens, which enable tumor cell survival and proliferation in the absence of essential amino acids (EAAs).[66-68] In terms of mechanism, KRAS expedites CD138 membrane recycling through activation of the MAPK-PSD4-ARF6 axis, which provides another potential therapeutic target worthy of further consideration.[62]

TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) is a dynamic network that includes malignant cells, immune cells, fibroblasts, and extracellular matrix components and influences the progression of tumors and the therapeutic response (Figure 2). Dey *et al*.[69] reported that oncogenic KRAS could mediate metabolic reprogramming in PDAC by utilizing cytokines from the TME. Murine *Kras* mutation in PDAC drives cell-autonomous expression of type I cytokine receptor complexes (IL2rγ-IL4rα and IL2rγ-IL13rα1) that are capable of receiving Th2 cytokines (IL4 or IL13) produced by invading Th2 cells in the TME. The ligand-induced activation of cytokine receptor signals stimulates cancer cell–intrinsic MYC transcriptional upregulation to enhance glycolysis.[69]

In response to the tumoral hypoxic microenvironment caused by poor vascularization and high interstitial pressure, activated Kras in mouse tumor cells stabilizes Hif1a and Hif2a to increase carbonic anhydrase 9 (Ca9) levels, which next commands the pH value and glycolysis to maintain cell survival.[70] The progression of PDAC is also driven by surrounding endocrine cells in the pancreas.[71] The abnormal expression of cholecystokinin (*Cck*) in β cells from obese mice promotes oncogenic *Kras*-driven pancreatic tumorigenesis.[71]

Recently, Tang's group found that KRASG12D protein could be released from cancer cells succumbing to autophagy-dependent ferroptosis upon oxidative stress. Extracellular KRASG12D protein is then taken up by macrophages via an AGER-dependent mechanism, which drives macrophages to switch to an M2-like protumor phenotype via STAT3-dependent fatty acid oxidation.[72]

PERSPECTIVES IN EARLY DIAGNOSIS

Positron emission tomography (PET) is a nuclear medicine procedure based on the measurement of positron emission from radiolabeled tracer molecules.[73] Given the hallmark of enhanced glucose metabolism in cancer, the most common metabolite-related radiotracer in use today is 18 fluorine-fluorodeoxyglucose (18F-FDG), a radiolabeled glucose analog. Imaging with 18F-FDG PET has been widely used to determine the sites of abnormal glucose metabolism and can be used to characterize and localize many types of tumors.[74, 75] Unfortunately, the current 18F-FDG PET imaging has limitations in detecting early-stage or small metastatic lesions of pancreatic cancer.[76-80] Many efforts have been made toward developing more tumor-specific radiotracers for PET imaging. For example, according to the extreme demand of glutamine for tumor cells, 18F-labeled glutamine analogs are currently being developed and have demonstrated their efficiency in preclinical animal models.[81-84]

Metabolic reprogramming is an early event in pancreas carcinogenesis initiated by *KRAS* mutation, suggesting a rationale for the development of related methods for the early diagnosis of PDAC. To develop more selective radiotracers for early diagnosis, intensive studies are required to understand the cellular metabolic changes in the early stages of pancreatic malignant cells and even in precancerous cells (*e.g.*, ADM and PanINs). However, our current understanding of metabolic reprogramming at the early stage of PDAC is based primarily on *in vitro* cell models and transcriptome or scRNA-seq analyses. Due to technical limitations, it is difficult to measure the metabolome and track metabolites using *in vivo* models. With technological innovations (*e.g.*, single-cell metabolomics) in the field of metabolic research, more in-depth studies would help us better understand how oncogenic KRAS drives metabolic reprogramming to initiate pancreatic cancer.

THERAPEUTIC OPPORTUNITIES

Oncogenic *KRAS* mutations are present in the overwhelming majority of patients with PDAC, which makes KRAS naturally the most valuable target. Unfortunately, direct targeting of KRAS has been demonstrated to be ineffective, mainly because the activation and signaling of RAS proteins are primarily accomplished through protein-protein interactions. Such interfaces have traditionally been difficult to target with small molecules due to their lack of well-defined binding pockets.[85, 86] Recent efforts have led to the development of pharmacological inhibitors targeting the KRASG12C mutant, which have shown promising results in early clinical trials.[87,88] However, targeting KRASG12D remains a major challenge. Therefore, there is an urgent need for therapeutics targeting KRASG12D mutants, especially for PDAC. Unfortunately, targeting downstream effectors of oncogenic KRAS, such as the RAF-MEK-ERK pathway and PI3K-AKT-mTOR pathway, has been demonstrated to be ineffective.[86, 89-92] Up to now, several targeted therapies against the mitogen-activated protein kinase kinase (MEK), extracellular-signal regulated kinase (ERK), phosphoinositide 3-kinases (PI3Ks), and mechanistic target of rapamycin (mTOR) signaling pathways have been demonstrated to be effective in impeding cell proliferation and tumor progression in human PDAC cell lines and mouse models. However, none of these has any impact on survival benefits according to clinical trials.[92-95]

Recently, metabolic enzyme inhibitors have received increasing attention for their therapeutic potential. Of note, inhibitors targeting isocitrate dehydrogenase (IDH) mutations have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of acute myeloid leukemia carrying IDH2 and IDH1 mutations, representing the first breakthrough in the translational research of tumor metabolism.[96, 97] In addition, metabolic targets such as glucose transporter protein type 1 (GLUT1), lactate dehydrogenase-A (LDHA), and glutaminase (GLS) have also shown antitumor effects in other tumors, such as breast cancer and ovarian cancer.[98-101]

The novel insights into the metabolic alterations associated with *KRAS* mutations provide exciting possibilities for targeting these poor-prognosis cancers. Many metabolic inhibitors have shown significant inhibitory effects on PDAC in preclinical culture and animal models; however, none has been approved for patients with PDAC.[102-105] Herein, according to the results of existing clinical trials, as well as drug efficacy and resistance issues, this field needs more in-depth exploration in the future.

CONCLUSION

Here, we summarized and highlighted recent advances in understanding how oncogenic *KRAS* mutations reprogram cellular metabolism in PDAC. Oncogenic KRAS activation alters glucose uptake, glycolytic flux, glutamine usage, nucleotide synthesis, lipolysis, and lipogenesis processes in PDAC to meet specific demands for energy metabolites

during PDAC rapid progression. Moreover, to adapt to the scarcity or imbalance of nutrient availability, oncogenic KRAS is also able to activate metabolic scavenging pathways, such as autophagy and macropinocytosis. Elucidating the role of oncogenic KRAS in metabolic reprogramming provides novel therapeutic interventions for PDAC.

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Conflicts of Interest

None declared.

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