HIPK2 sustains the oncogenic activity of mutant KRAS in Colorectal Cancer

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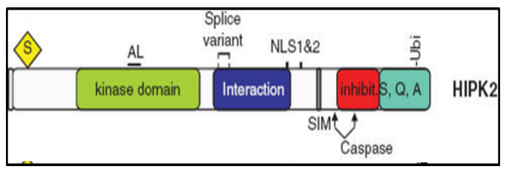
ABSTRACT

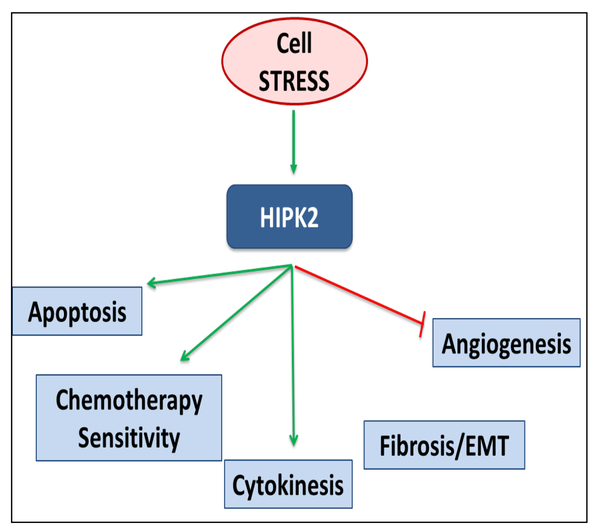
Activating mutations of the *KRAS* gene are found in about 40% of colorectal cancers (CRC). Clinical observations suggest a causal role for KRAS mutations in disease progression and in governing therapeutic responses to targeted therapy. In particular, studies have shown that monoclonal antibodies that target epidermal growth factor receptors (EGFRs) provide a survival benefit only for CRC with wild-type KRAS. Since the effectiveness of current treatments in patients harboring KRAS mutations is limited, novel therapeutic strategies are urgently needed.

HIPK2 is an evolutionary conserved kinase involved in different cellular processes such as development, cell division and survival.

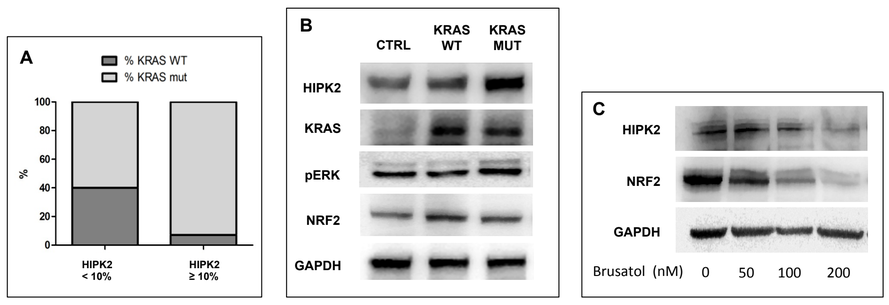
A retrospective study conducted on CRC samples from our Institute showed that HIPK2 expression levels increase with tumor stage and invasion. In particular, we found a strong association between elevated HIPK2 expression and a high-risk of recurrence. Such association is reversed when the analysis is performed at early stages on patients which received adjuvant therapy, suggesting that HIPK2 high expression may favor the response to therapy. Additionally, and more interestingly, we identified an association between higher HIPK2 expression and KRAS mutations. Experiments performed in CRC cells lines have shown the existence of a causal relationship between mutated KRAS and increased HIPK2 expression. Moreover they unveiled a functional relevance for HIPK2 in the activation of the pathways downstream KRAS. Finally, preliminary data suggest that modulation of HIPK2 expression may alter the cellular response to KRAS pathway inhibitors.

Our data strongly suggest that HIPK2 might contribute to the pathogenesis of CRC, possibly in concert with mutated KRAS. Our current efforts are therefore aimed at identifying the molecular determinants of the relationship between mutated KRAS and HIPK2 and at verifying its use as therapeutic target. Moreover, since the *HIPK2* gene family is known to produce abundant circular RNAs (circRNAs) which can be easily detected in body fluids, we will verify the possibility of using circHIPK2 as a biomarker, in particular in association with KRAS mutations.

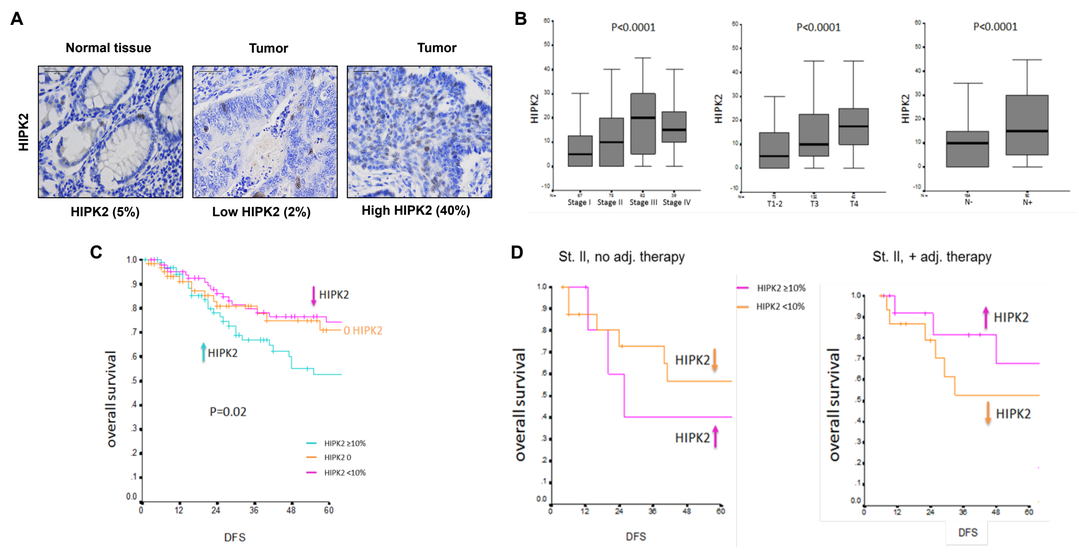




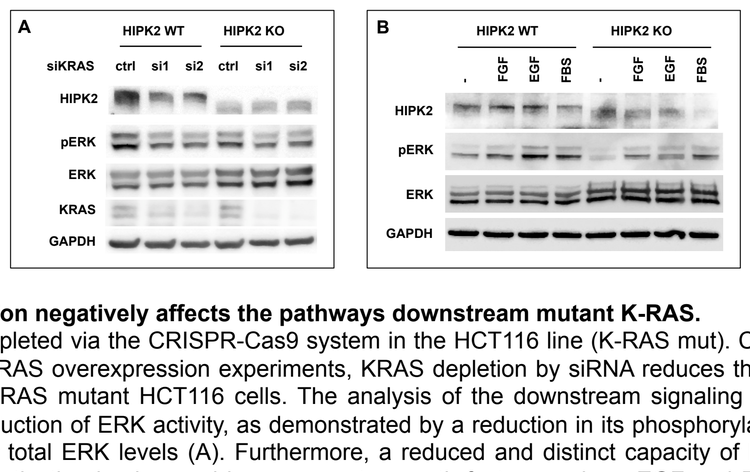
HIPK2: A multi-talented protein which integrates signals from multiple pathways.
HIPK2 (homeodomain-interacting protein kinase 2) is an evolutionarily conserved serine/threonine kinase. HIPK2 acts as a co-regulator of transcription factors and modulates many different cellular processes such as growth, development, morphogenesis and cell death. HIPK2 binds and phosphorylates an extensive variety of targets, including transcriptional regulators, chromatin modifiers and signal transducer. HIPK2 is also involved in the regulation of gene transcription in response to DNA damage as triggered by UV light, ionizing radiation, and chemotherapeutic drug treatment and modulates the activity of several proteins related to apoptosis, including the tumor suppressor p53 and its family members. HIPK2 has also been implicated in the activation of multiple downstream signaling pathways including those affected in CRC such as Wnt/β-Catenin and TGF-β/Smad pathways.



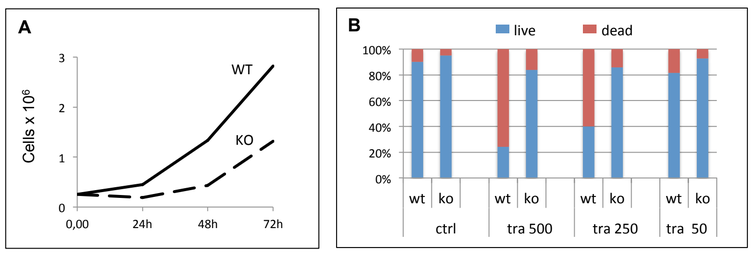
Higher HIPK2 expression associates with mutant KRAS.
HIPK2 mutational status determined by exome sequencing in a group of CRC biopsies is consistent with COSMIC data and shows a very low frequency (<5%) of HIPK2 mutations in the samples analyzed (n=80). This would exclude that different patterns of HIPK2 expression might be due to mutations. CRC samples where then analyzed by Next Generation Sequencing (NGS) for a panel of 50 cancer related genes (n=120). A substantial relationship emerged between HIPK2 expression and KRAS mutations. In particular, KRAS mutation are prevalently found in tumors with HIPK2 expression ≥ 10% (A). In order to test whether the presence of mutated KRAS may directly affect HIPK2 expression levels, an expression construct for K-RAS mut was transfected in CRC lines with K-RAS WT (RKO cells). The data show that indeed, K-RAS mut induces an increase in HIPK2 expression levels (B).
The transcription factor NRF2 is one of the main regulators of the oxidative damage response. It has been shown that KRAS induces an increase in the expression of NRF2, which contributes to tumorigenicity and chemo-resistance. More recent studies have shown that HIPK2 is a direct target of NRF2 and is necessary for its activity. Our preliminary data show that NRF2 expression is modulated by KRAS concomitantly with HIPK2 (B) and that the NRF2 inhibitor Brusatol negatively affects HIPK2 expression (C). Further efforts will be directed to evaluate whether NRF2 can mediate the functional relationship between K-RAS and HIPK2 in the CRC context.



HIPK2 expression levels increase with tumor stage and invasion and associate with risk of recurrence.
Tissue Microarrays (TMAs) of cancer samples from a retrospective series of 280 stage I-IV CRC patients with known follow up have been analyzed for HIPK2 expression by IHC. Compared to normal tissue where the nuclear staining of HIPK2 is detectable in around 5% of colon villus cells, in different tumors HIPK2 is expressed at variable levels ranging from 0% to around 45% of cells (A). Interestingly, data analysis clearly shows that the percentage of HIPK2 expressing cells increases with tumor stage and invasion (B). Besides, a direct association between the percentage of tumor cells expressing HIPK2 and the risk of recurrence was found (C). Such association is reversed when the analysis is performed at early stages on patients which received adjuvant therapy (D), suggesting that HIPK2 expression may favor the response to therapy.



HIPK2 depletion negatively affects the pathways downstream mutant K-RAS.
HIPK2 was depleted via the CRISPR-Cas9 system in the HCT116 line (K-RAS mut). Consistent with what observed in KRAS overexpression experiments, KRAS depletion by siRNA reduces the expression levels of HIPK2 in KRAS mutant HCT116 cells. The analysis of the downstream signaling pathways of KRAS revealed a reduction of ERK activity, as demonstrated by a reduction in its phosphorylation (pERK) levels, despite higher total ERK levels (A). Furthermore, a reduced and distinct capacity of HIPK2-null HCT116 cells in ERK activation is observed in response to growth factors such as EGF and FGF, which bind the respective tyrosine kinase receptors upstream KRAS (B).



HIPK2-null cells display a reduced proliferation rate and a decreased resistance to MEK inhibition.
In agreement with a reduced activation of KRAS/ERK signaling, HIPK2-KO HCT116 cells display a significantly reduced proliferation rate as shown by the growth curve (A). However, when compared to the WT controls, HIPK2-KO HCT116 cells are more resistant to treatment with Trametinib (tra), a MEK-specific inhibitor currently used in the clinic (B), this again suggesting that HIPK2 deprivation and MEK-ERK inhibition might impinge on the same pathway.