Emerging evidence indicates that Raf-1 kinase inhibitor protein (RKIP) is a critical tumor and metastasis suppressor and promotes drug-induced apoptosis in different cancer types. We and others have previously shown that RKIP is very frequently down-regulated, both at mRNA and protein level, in hepatocellular carcinoma (HCC). Beside other activities, RKIP is as an inhibitor of MAPK and NF-κB signalling pathways, which are hyper-activated in HCC. However, different mechanisms examined in human HCC cell lines (HA22T/VGH, HepG2 and Hep3B) and clinical HCC samples did not appear to be responsible of RKIP down-regulation. In particular, by sequencing the whole RKIP gene, we did not find any gene variant that might account for the low RKIP levels. Not even RKIP gene methylation could explain the result. Further, though different elements had suggested that RKIP may be a target repressed by miR-224, a miRNA that is frequently and specifically up-regulated in HCC, our results excluded that this occurs, at least in the HCC cell lines. Finally, factors like Snail, EZH2 and HDAC have been implicated in the RKIP down-regulation present in breast and prostate tumors; though some our results from the cell lines do not support that they play such a role in HCC, this aspect is worthy of further study (Poma et al., 2012).

On the other hand, more recent results have indicated that proteasomal and NF-κB activities may play a significant role in RKIP inhibition, since we have observed that proteasome inhibition by MG132 or bortezomib increases RKIP mRNA and protein levels in the HCC cell lines. Accordingly, treatment of HA22T/VGH cells with MG132 caused a reduction of their invasiveness ability, which was evaluated by Matrigel invasion assay. Proteasome inhibition may act by impeding a NF-κB-mediated repression of RKIP gene transcription, since treatment of the HA22T/VGH cell line with the NF-κB inhibitor DHMEQ was also able to increase RKIP mRNA and protein levels. Nevertheless, we can not exclude that inhibition of degradation of RKIP protein may also account for the effect of MG132 or bortezomib and are testing this possibility. In conclusion, although the causes of RKIP down-regulation in HCC remain incompletely understood, we have now at our disposal some pharmacological approaches able to restore the relevant antitumor function of the factor in HCC. This paves the way for further interesting investigations.