HIPK2 and cancer cell resistance to therapy

“HIPK2 may act as a tumor suppressor in human tissues, but unfortunately, data concerning HIPK2 regulation in human tumors *in situ* are sparse.”

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Within the last decade, members of the homeo-domain-interacting protein kinase (HIPK) family of serine/threonine kinases have been found to regulate cell-fate decisions at multiple levels. The best-studied family member is HIPK2, which was originally identified as a transcriptional corepressor of homeodomain transcription factors such as NK-3 [1].

HIPK2 is now considered as a central switch in the targeting of cells towards apoptosis upon genotoxic stress by phosphorylating tumor suppressor p53 at Ser46 [2,3]. This modification promotes p53 acetylation by CREB-binding protein and redirects the transcription factor p53 to proapoptotic target genes such as *PUMA* and *Noxa*.

This pathway has received much attention during the last few years, and it has been demonstrated that HIPK2 facilitates p53-dependent apoptosis in tumor cell lines in response to UV- and ionizing radiation [4], as well as to a variety of chemotherapeutic agents, namely cisplatin [5], adriamycin [6] and roscovitine [7].

However, importantly, the proapoptotic functions of HIPK2 are not limited to the p53 pathway. HIPK2 also plays a role in TGF- β signaling, where it is important for JNK activation and JNK-mediated apoptosis [8]. Moreover, it seems that HIPK2 can facilitate the degradation of the antiapoptotic transcriptional corepressor C-terminal binding protein (CtBP). This degradation is dependent on Ser422 phosphorylation of CtBP, which may occur directly via HIPK2 itself [9], or indirectly via JNK [10], and, again, takes place after cytotoxic insults, such as UV irradiation or cisplatin treatment of tumor cell lines. Very recently, HIPK2 has also been reported to antagonize LEF/ β -catenin signaling, possibly by facilitating the degradation of β -catenin via an as yet unknown mechanism, which might antagonize

the growth of colorectal carcinoma and hepatoma cells. This β -catenin degradation may be in part responsible for the capacity of HIPK2 to downregulate VEGF expression, and thus to reduce neoangiogenesis. Intriguingly, HIPK2 is bound and activated by Axin, a scaffold protein found to enhance β -catenin degradation, and an axin-HIPK2-p53 complex was reported to activate proapoptotic p53 target genes [11]. Taken together, these findings suggest that HIPK2 may act as a tumor suppressor in human tissues through different pathways, preventing aberrant proliferation of severely damaged cells by driving them into apoptosis.

Unfortunately, data concerning HIPK2 regulation in human tumors *in situ* are sparse. The earliest study that deals with HIPK2 levels in human cancer reports downregulation of HIPK2 in many thyroid and breast carcinomas in comparison with the respective normal tissues [12]; however, these interesting findings still need to be extended by functional studies. Several years later in 2007, two missense mutations were identified within the HIPK2 speckle retention signal in human acute myeloblastic leukemias (AMLs) and in myelodysplastic syndrome, which led to an aberrant diffuse distribution of HIPK2 within the cell nucleus [13]. The disturbed localization of HIPK2 led to impaired transcription by the AML1 and p53 transcription factors. However, aberrantly expressed HIPK2 was only found at a very low frequency (two out of 130 samples), and thus, a putative causative role of HIPK2 in leukemic cell transformation remains to be shown. However, interestingly, in a very recent publication, Wee *et al.* report that a potentially leukemogenic fusion protein consisting of PEBP2 β (also termed CBF β) and SMMHC, relocalizes HIPK2 to filamentous structures in the cytoplasm, thereby inhibiting

HIPK2-dependent phosphorylation of p300 in the nucleus [14]. Similarly, Pierantoni *et al.* demonstrated in 2007 that the expression of the oncogenic high-mobility group A1 (HMGA1) protein in breast carcinoma is well correlated with increased cytoplasmic HIPK2 staining and decreased apoptosis in these tumors, likely via decreased p53 Ser46 phosphorylation [15]. In addition, in 2007, Wei *et al.* published an interesting study in which *HIPK2*^{-/-} and even *HIPK2*^{+/-} mice exhibited higher sensitivities to skin tumor formation in a two-step carcinogenesis model, indicating that in normal mouse skin, HIPK2 indeed suppresses malignant cell growth [16]. With regard to HIPK2 levels in tumors, it is particularly interesting to note that the malignant phenotype already appeared in heterozygous mice, which supports the notion that HIPK2 dosage is crucial in suppressing aberrant cell growth, and suggests HIPK2 as a haploinsufficient tumor suppressor.

However, by contrast, HIPK2 does not seem to suppress, but rather to promote tumor growth in cervical cancer, since it was strongly expressed in more than 75% of cervical cancer samples as compared with only 10% of normal control tissues in another very recent study [17]. The expression of HIPK2 in this cancer type apparently correlated with tumor progression. Moreover, another recently published observation of Deshmukh *et al.* suggests that in certain cancers, enhanced HIPK2 expression is actually advantageous for tumor progression [18]: HIPK2 is overexpressed in approximately two-thirds of pilocytic astrocytomas, which are relatively benign gliomas that primarily occur in childhood and early adolescence, and to a certain (albeit much lower) extent in other glioma types [18].

“HIPK2 may well turn out to be a promising target for tumor therapy.”

Unfortunately, neither of these studies went on to investigate the relationship between HIPK2 expression and sensitivity towards chemo- or radiotherapy. Therefore, until recently, it remains to be shown that the HIPK2 status does indeed have an impact on the outcome of cancer therapy. Owing to the functional promiscuity of HIPK2, it is entirely possible that HIPK2 deficiency or proficiency will differentially influence the sensitivity to irradiation and cytotoxic chemicals in p53-positive and -negative malignancies. In p53-expressing cells, HIPK2 activation would be

expected to potentially enhance apoptosis in response to treatment. Thus, we would expect that in p53-positive cancer, HIPK2 expression would be selected against. Indeed, Puca *et al.* reported earlier this year that overexpression of HIPK2 in p53 wild-type, chemoresistant ovarian cancer cells can render these cells sensitive to therapy by mediating p53 phosphorylation [19]. Puca *et al.* also presented a second study suggesting that HIPK2 is required for native folding of the p53 protein in colon cancer cells; surprisingly, HIPK2 action can be replaced by the addition of zinc to the cells, possibly suggesting a therapeutic function of zinc ions in p53-proficient malignancies [20]. However, in p53-negative cancer, HIPK2 may still promote apoptosis by derepressing CtBP target genes or by inducing JNK, but recent evidence from *HIPK2* knockout mice and the studies of Al-Beiti *et al.* and Deshmukh *et al.* suggest that HIPK2 may also play a role in cell proliferation and survival in some cell types [17,18]. Indeed, this was shown by using the glioma cell-line U87, which exhibits enhanced cell growth in a colony formation assay when transfected with wild-type, but not kinase dead HIPK2 [18]. Furthermore, a prosurvival role of HIPK2 in dopaminergic neurons was also reported using a HIPK2 knockout mouse model [21]. Thus, it is conceivable that some cancer patients might even benefit from HIPK2 inhibition, particularly in cases of elevated HIPK2 levels in the absence of p53.

The regulation of HIPK2 levels has been subject to intense investigation in previous years. We and others have shown that HIPK2 is an unstable protein that is degraded via the proteasomal pathways. In particular, the ubiquitin ligases WSB-1 [22] on the one hand and Siah-1 and -2 on the other hand have been shown to target HIPK2 for degradation, a process that is inhibited by cytotoxic stress, and in the case of Siah-1, via the activation of the DNA damage checkpoint kinases ATM and/or ATR [23]. This implies that in order to block HIPK2 function, cancer cells may not only up- or downregulate HIPK2 on the mRNA level, but may induce its stabilization or degradation by modulating its ubiquitin ligases. Indeed, we could demonstrate that depletion of Siah-1 leads to an enhanced apoptosis induction [23]. Intriguingly, it has been shown that Siah proteins are active in hypoxia, which is often present in solid tumors [24]. *Siah-1* also appears to be a p53 target gene, implying that Siah-1 is part of a negative-feedback mechanism that

helps cells to survive after p53 induction. Concordantly, p53-deficient tumor cell lines indeed show elevated HIPK2 protein levels [23], suggesting that HIPK2 may be stabilized in p53-deficient cancer.

Interestingly, a recent study suggests that Siah proteins are essential mediators of oncogenic ras-induced cell transformation and tumorigenesis, but whether HIPK2 degradation is important in this transformation has not yet been determined [25].

In summary, HIPK2 may well turn out to be a promising target for tumor therapy. Exogenous activation of HIPK2 may sensitize particularly p53-proficient cancer cells to conventional chemotherapy, whereas inhibition of HIPK2 in tumors of neuronal origin may inhibit growth or even induce apoptosis in these cells. Importantly, HIPK2 levels may constitute a useful prognostic marker for the outcome of

conventional chemotherapy, but much work remains to be carried out in order to systematically characterize the cancer entities in which HIPK2 modulates cell death or survival.

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