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Comments

Growth inhibitory effect of Hcc-1/CIP29 is associated with induction of apoptosis, not just with G_2/M arrest

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We read the recent article by Leaw et al. 'Hcc-1 is a novel component of the nuclear matrix with growth inhibitory function' [1] with great interest. Hcc-1 was cloned from a hepatocellular carcinoma [2], and turned out to be identical to the cytokine-inducible 29-kDa protein (CIP29) cloned by us [3]. However, the function of the Hcc-1/CIP29 protein is not yet understood. In their article, Leaw et al. showed that Hcc-1 binds to two DEAD-box RNA helicases, BAT1 and DDX39, and found that over-expression of Hcc-1 in HEK293 cells results in an inhibition of cell growth that is associated with G_2/M arrest and inhibition of S phase [1]. These findings are very important in trying to understand the function of Hcc-1/CIP29; however they are inconsistent with several of our findings, and this is of relevance in the interpretation of mechanisms. First, Leaw and colleagues did not detect Hcc-1 transcript using RT-PCR in the majority of the normal human tissues and cancer tissues in contrast to our study, in which we detected CIP29 using Northern analysis in almost all human tissues examined, including cancer tissues [3]. They also claim that HEK293 cells do not express Hcc-1 mRNA and protein; however our unpublished observation, again using Northern analysis, clearly indicates that CIP29 mRNA is expressed in HEK293 cells, although apparently not abundantly. Failure to detect mRNA by RT-PCR in their experiments may be due to the amount of mRNA used or the number of PCR cycles (they used 26 cycles), which may not be optimal for detection of Hcc-1/CIP29 transcript. Absence of Hcc-1/CIP29 protein in HEK293 cells could be due to sensitivity of the antibody or the efficiency of the protein extraction method, since CIP29 is a nuclear protein [2, 3]. In this regard, longer exposure of the Western blot may allow detection of this protein. Second, Leaw et al. reported that over-expression of Hcc-1 in HEK293 cells inhibited cell proliferation after culture for 96 h in the presence of FBS, which is similar to our findings that over-expressing a CIP29-EGFP fusion protein in HEK293 cells significantly decreases viable cell number compared to cells expressing EGFP alone after serum withdrawal over 96 h. However, in contrast to their observation that Hcc-1 expression induces G_2/M arrest and decreases the number of HEK293 cells in S phase after culture for 48 h in the presence of 10% FBS, we observed an increase of cells in S+G_2/M phase following FBS deprivation, which was not due to a selective increase in G_2/M [3]. Furthermore, instead of Hcc-1 inducing G_2/M arrest, we found that over-expression of CIP29-EGFP protein significantly enhanced apoptosis measured by hypodiploid DNA content compared to cells expressing EGFP alone [3]. We would be very interested to know if the authors examined apoptosis in their cells in the presence of FBS; they do not report this in their paper. The difference between our and their results could be due to experimental conditions. We examined cell proliferation, the cell cycle and apoptosis in the absence of FBS using a CIP29-EGFP construct in HEK293 cells, while Leaw et al. investigated cell proliferation and the cell cycle in the presence of FBS using full-length Hcc-1. Although we did not examine cell proliferation at 96 h, proliferation of HEK293 cells transfected with CIP29-EGFP was equivalent to that of cells transfected with EGFP alone for 72 h in the presence of 10% FBS, which

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does not contradict the data shown in figure 6 of their report. To rule out a functional difference between CIP29-EGFP protein and full-length CIP29, we also transfected CIP29-EGFP or CIP29-IRES-EGFP constructs into TF-1 cells. As in HEK293 cells, both constructs resulted in growth inhibition to the same extent following deprivation of FBS, suggesting that the functions of the CIP29-EGFP fusion protein and full-length CIP29 are equivalent. Therefore, Hcc-1/CIP29 appears to inhibit cell proliferation by inducing G_2/M arrest in the presence of FBS, but inhibits cell proliferation by inducing apoptosis without arresting cells in G_2/M following deprivation of FBS. This suggests that Hcc-1/CIP29 has multiple functions in regulating cell fate. The inhibitory effect on cell proliferation and induction of apoptosis or G_2/M arrest by Hcc-1/CIP29 is somewhat puzzling since CIP29 is up-regulated by hematopoietic growth factors, which normally enhance cell survival, the cell cycle and proliferation [3], and is also up-regulated in several cancer cells, which possess enhanced survival and proliferation characteristics [2, 3]. Induction of Hcc-1/CIP29 by growth factors may, therefore, be part of a negative feedback loop regulating cell proliferation.

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3 Fukuda S, Wu D. W., Stark K. and Pelus L. M. (2002) Cloning and characterization of a proliferation-associated cytokine-inducible protein CIP29. Biochem. Biophys. Res. Commun. **292:** 593–600