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EXTRA VIEW

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Tumor cells with *KRAS* or *BRAF* mutations or *ERK5/MAPK7* amplification are not addicted to ERK5 activity for cell proliferation

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ABSTRACT

ERK5, encoded by *MAPK7*, has been proposed to play a role in cell proliferation, thus attracting interest as a cancer therapeutic target. While oncogenic RAS or BRAF cause sustained activation of the MEK1/2-ERK1/2 pathway, ERK5 is directly activated by MEK5. It has been proposed that RAS and RAF proteins can also promote ERK5 activation. Here we investigated the interplay between RAS-RAF-MEK-ERK and ERK5 signaling and studied the role of ERK5 in tumor cell proliferation in 2 disease-relevant cell models. We demonstrate that although an inducible form of CRAF (CRAF:ER*) can activate ERK5 in fibroblasts, the response is delayed and reflects feed-forward signaling. Additionally, oncogenic KRAS and BRAF do not activate ERK5 in epithelial cells. Although KRAS and BRAF do not couple directly to MEK5-ERK5, ERK5 signaling might still be permissive for proliferation. However, neither the selective MEK5 inhibitor BIX02189 or ERK5 siRNA inhibited proliferation of colorectal cancer cells harbouring $KRAS^{G12C/G13D}$ or $BRAF^{V600E}$. Furthermore, there was no additive or synergistic effect observed when BIX02189 was combined with the MEK1/2 inhibitor Selumetinib (AZD6244), suggesting that ERK5 was neither required for proliferation nor a driver of innate resistance to MEK1/2 inhibitors. Finally, even cancer cells with *MAPK7* amplification were resistant to BIX02189 and ERK5 siRNA, showing that ERK5 amplification does not confer addiction to ERK5 for cell proliferation. Thus ERK5 signaling is unlikely to play a role in tumor cell proliferation downstream of KRAS or BRAF or in tumor cells with ERK5 amplification. These results have important implications for the role of ERK5 as an anti-cancer drug target.

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Introduction

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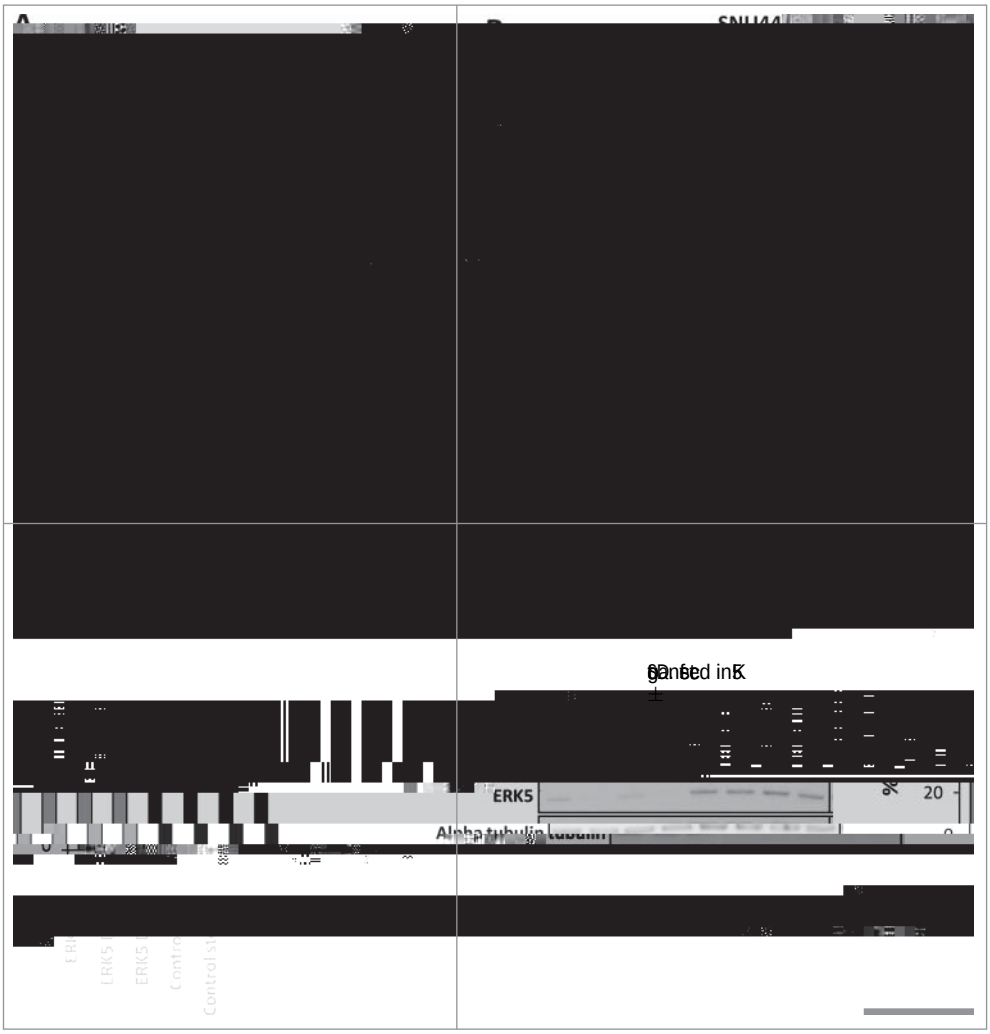

Figure 7. The hetatocellular carcinoma cell line SNU449 expresses high levels of ERK5 but is not dependent on MEK5-ERK5 signaling for proliferation. (A) Subconfluent cultures of BT474 and the liver hepatocellular carcinoma cell line SNU449 harbouring an amplification containing the ERK5 gene were maintained in 10% FBS. Cells were lysed, whole cell lysates were separated by SDS-PAGE and immunoblotted with the antibodies indicated. (B) Subconfluent cultures of SNU449 cells were maintained in 10% FBS then treated with increasing concentrations of BIX02189 (100 nM to 30 μM) for 24 or 48 hours, and DNA synthesis was assayed by [^{3}H]thymidine incorporation; the results are presented as an average of 3 experiments $\pm$ SD. Alternatively, cells were transfected as in Fig. 2(C), 6h post-transfection cell were treated with increasing concentrations of BIX02189 (100 nM carci9(7.6(to5t-tran8589131.02750TD(m)TJF51Tf.690TDtures))-25ained)-254(with)-251.-25ai. C0lls to7239TD1.733 con

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Disclosure of potential conflicts of interest

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