

Cancer Therapy by Activation of Human Checkpoint Kinase 2



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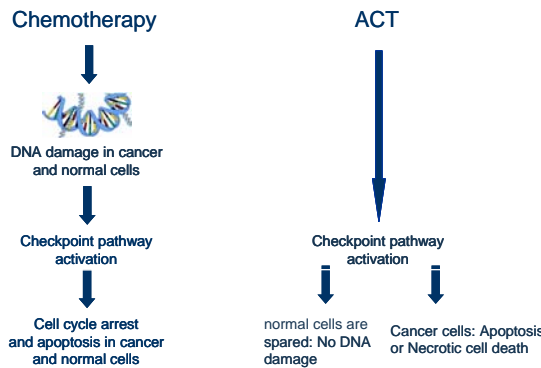
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Abstract

Chromosomal aberrations in tumors often result in amplification, overexpression, or overactivation of oncogenic signaling pathways that can lead to unrestrained proliferation, a hallmark of cancer cells. DNA damage checkpoint pathways were found to act as an anti-cancer barrier in early human carcinogenesis, however, this endogenous defense against malignant transformation is compromised at advanced stages of tumorigenesis. The goal of this study was to determine if direct activation of DNA damage checkpoint pathways, in the absence of *de novo* DNA damage, could disrupt cancer maintenance and lead to killing of cancer cells. We found that activation of checkpoint kinase 2 (Chk2) by expression-induced autophosphorylation led to cell death in multiple cancer cell lines, independent of p53 status. We also examined the anti-tumor activity of Chk2 in a human colon cancer xenograft model using a tetracycline inducible Chk2 cell line. Activation of Chk2 *in vivo* showed potent growth inhibition of pre-established tumors derived from a Chk2-inducible Dld1 colon cancer cell line. Furthermore, two classes of small molecules were identified that showed potent and sustained activation of Chk2 as measured by Chk2 phosphorylation. Selective toxicity, the guiding principle for chemotherapy, has typically been achieved by inhibiting a biological pathway. Here we demonstrate that we can achieve selective toxicity through activation of a biological pathway and that DNA damage checkpoint pathways may protect against cancer after malignant transformation.

Can Cancer be Treated by Activating Genome Surveillance (Checkpoint) Pathways?



Checkpoint Pathway Activators against Cancer

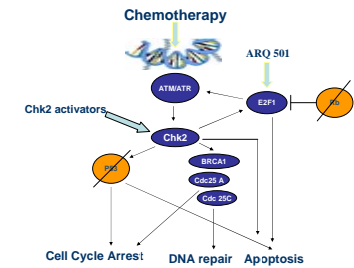


Figure 4

Figure 1

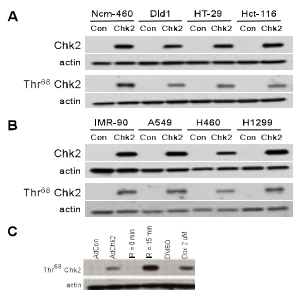


Figure 1. Chk2 expression and activation in tumor cell lines
Colon cell lines (A) or lung cell lines (B) were infected with AdChk2 at a multiplicity of infection (MOI) of 25. Cells were harvested and extracts made at 24 hours post infection (hpi). Western blot analysis was performed to determine levels of Chk2 and the phospho-threonine 68 form of Chk2. Actin blots are shown as protein loading controls. (C) Comparison of phospho-threonine 68 form of Chk2 in A549 cells infected with AdChk2, exposed to 2 Gy ionizing radiation for 15 min, or treated with 2 μM doxorubicin for 1 hour.

Figure 2

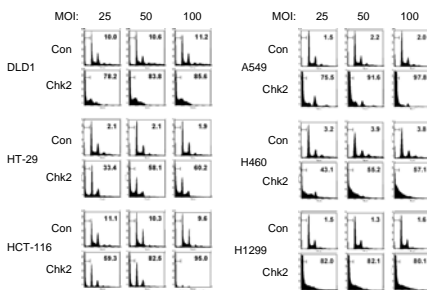


Figure 2. Induction of apoptosis in cancer cells by Chk2 activation
(A) Colon cell lines were infected with AdCon or AdChk2 at the indicated MOI. Cells were harvested and DNA stained with propidium iodide (PI) at 48 hpi. Number in each panel represents percentage of cells with sub-G1 DNA content. (B) Lung cell lines were infected with AdCon or AdChk2 at the indicated MOI. Cells were harvested and DNA stained with propidium iodide (PI) at 48 hpi. Number in each panel represents percentage of cells with sub-G1 DNA content.

Figure 3

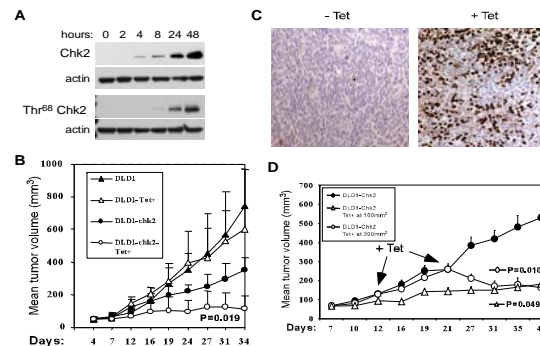


Figure 3. Chk2 expression inhibits tumor growth *in vivo*
(A) Western blot analysis was performed to determine levels of Chk2 and the phospho-threonine 68 form of Chk2 in Dld1-Chk2 inducible cell line following addition of 1.0 μg/ml tetracycline at time 0. Cells were harvested and extracts were made at indicated time points following addition of tetracycline to the growth medium. (B) DLD1-Control and DLD1-Chk2 cells were cultured in the presence or absence of tetracycline for 24 h. Then 4 x 10⁶ cells were harvested and injected subcutaneously into the flank region of female nude mice. The mice were fed water with (+) or without (-) 1.0 mg of tetracycline per ml. Tumor volumes (mm³) were monitored for 34 days. The change in tumor volume over a 34-day period is shown in the graph. Error bars represent standard deviations of tumor volumes. (C) Immunohistochemical staining in DLD1-Chk2 xenograft tumors with or without addition of tetracycline to the drinking water. (D) DLD1-Chk2 cells were injected subcutaneously into the flank region of female nude mice. When tumors reached volumes indicated by arrows (100 and 300 mm³), tetracycline was added to drinking water and tumor volumes (mm³) were monitored for 32 days. The change in tumor volume over a 32-day period is shown in the graph. Error bars represent standard deviations of tumor volumes.

Class 1 Chk2 Activators Class 3 Chk2 Activators

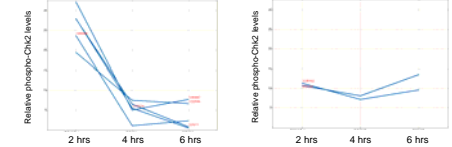


Figure 4. High content screening for compounds that activate Chk2. Compound libraries made by ArQule, Inc. were screened using high content fluorescence microscopy to detect the levels of phospho-Thr68 Chk2.

Conclusions

- Chk2 activation in cancer cells results in cell death, regardless of p53 status.
- We can achieve physiologically relevant levels of Chk2 activation in cancer cells.
- Chk2 activation inhibits tumor growth of xenografted colon cancer *in vivo*.
- We have identified multiple compounds that cause substantial and sustained Chk2 activation in cancer cells.