

ALCOHOL PROMOTES ACTIVATION OF CRUCIAL SIGNALING PROTEINS, AKT AND ERK 1/2, WHICH PROMOTE PROSTATE CANCER CELL SURVIVAL.

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ABSTRACT

An association between alcohol consumption and prostate cancer is not well-understood. Several studies suggest that there is not a correlation with alcohol use and prostate cancer risk while others indicate that high alcohol consumption and/or particular alcohol types may promote an increase risk in prostate cancer incidence. In addition to determining whether alcohol consumption plays a role in prostate cancer occurrence, it is also important to understand the effects of alcohol on prostate cancer cell signaling events. This study examined whether alcohol induces activation of key cell survival signaling proteins, AKT and ERK 1/2, in prostate cancer cells. Activation of these signaling proteins promotes cell survival by inhibiting programmed cell death, apoptosis. LNCap and DU145 prostate cancer cell lines were used in these examinations. Cells were incubated with physiological relevant alcohol concentration of 20mM for 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours. Epidermal Growth Factor (EGF) at 10ng/ml was used as a control stimulant because of its reproducible stimulation of AKT and ERK 1/2 activation. After each time point, the media without or with alcohol or EGF was removed and the cells were collected for further western blot and apoptosis analysis. Our results indicate that incubating physiological relevant alcohol concentrations with prostate cancer cells for short time periods, between 5 minutes and 1 hour, did not produce an affect on AKT and ERK1/2 activation, whereas incubation times greater than one hour promoted activation of AKT and ERK 1/2. The increase in activities of AKT and ERK1/2 as a consequence of alcohol incubation coincides with a subsequent decrease in the number of prostate cancer cells undergoing programmed cell death, apoptosis. This suggests that long exposure periods of prostate cancer cells to alcohol can be detrimental by enhancing cancer cell survival. This study demonstrates at a cellular level signaling pathways AKT and ERK 1/2 in prostate cancer cells are affected by alcohol exposure. These results indicate that a more effective treatment for patients suffering from alcoholism and prostate cancer may be combination therapy that incorporates targeting of these signaling pathways.

RESULTS

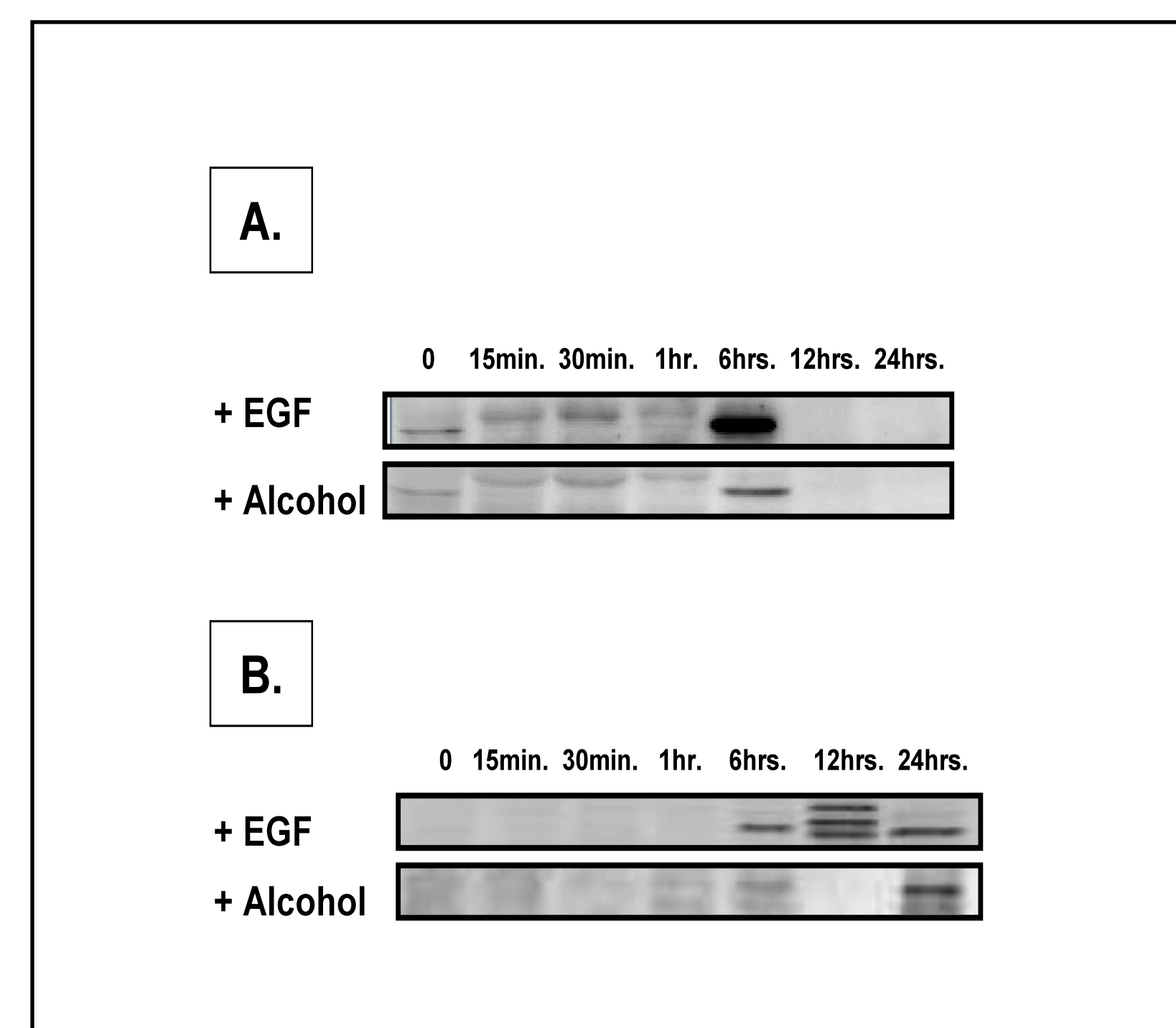


Figure 1 – Alcohol activates cell survival signal protein AKT in prostate cancer cells.
EGF and Alcohol stimulates AKT activation at 6 hours in LNCap (A) cells. Whereas, EGF stimulates AKT activation at 6 hours, 12 hours, and 24 hours in DU145 (B) cells and Alcohol stimulates AKT activation at 6 hours and more significantly at 24 hours in DU145 (B) cells.
LNCap (Panel A) and DU145 (Panel B) prostate cancer cells were incubated with 20mM Alcohol for 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours. In parallel plates, cells were stimulated with 10ng/ml EGF for the same time points before all plates of cells were lysed using boiling 2X Lamelli buffer with β -mercaptoethanol. Protein concentrations were determined using RC/DC BioRad protein assay for equal protein SDS-PAGE loading. Western blots were probed using antibodies for phospho-AKT (ser473) [Calbiochem, CA].

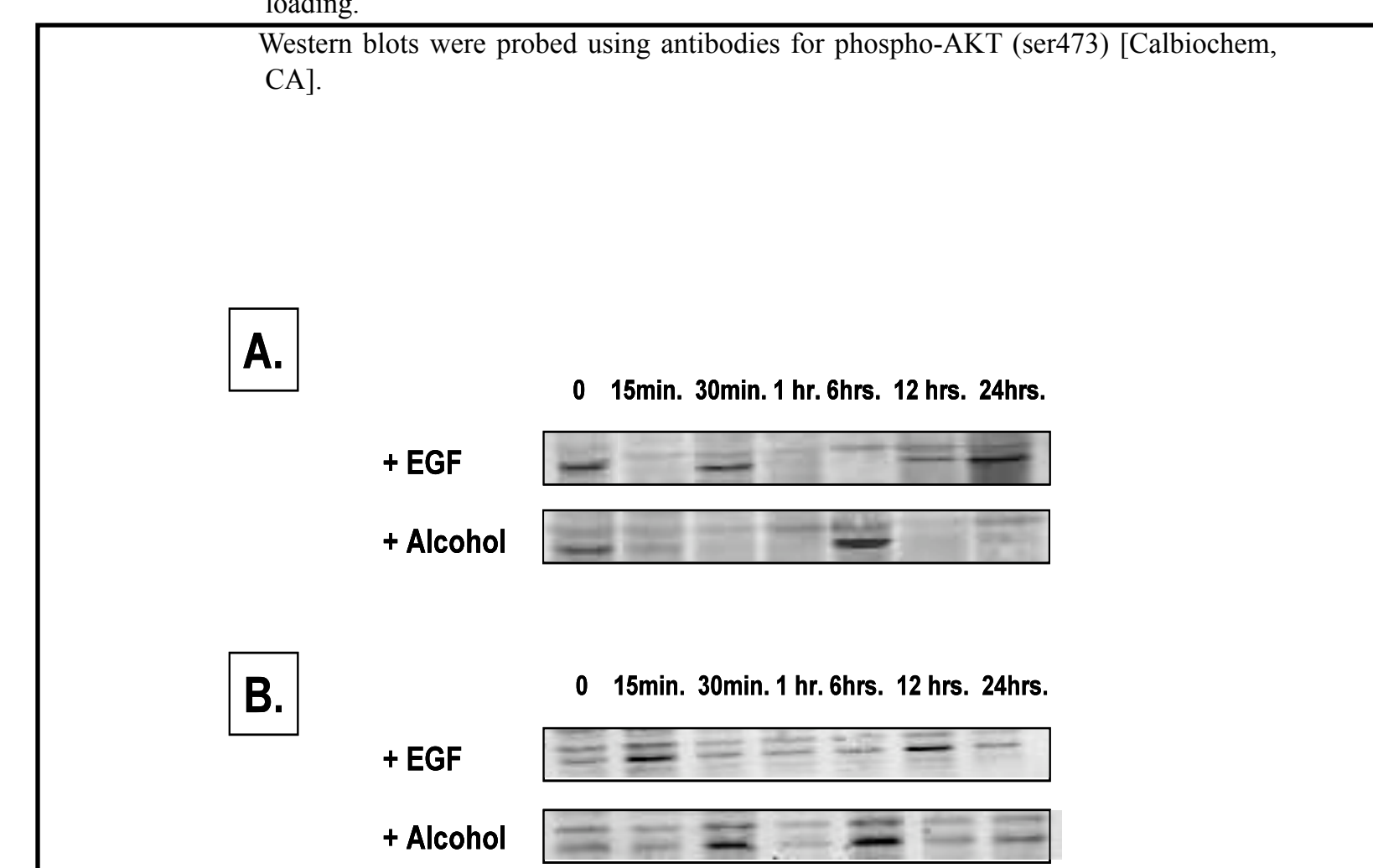


Figure 2 – Alcohol activates cell survival signal protein ERK 1/2 in prostate cancer cells.
In LNCap (A) prostate cancer cells EGF stimulates ERK activation at 30 minutes, 12 hours, and 24 hours, whereas Alcohol stimulates ERK activation at 6 hours. In DU145 (B) prostate cancer cells, EGF stimulates ERK activation at 15 minutes and 12 hours whereas Alcohol stimulates ERK activation at 30 minutes and 6 hours.
LNCap (A) and DU145 (B) prostate cancer cells were incubated with 20mM Alcohol for 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours. In parallel plates, cells were stimulated with 10ng/ml EGF for the same time points before all plates of cells were lysed using boiling 2X Lamelli buffer with β -mercaptoethanol. Protein concentrations were determined using RC/DC BioRad protein assay for equal protein SDS-PAGE loading. Western blots were probed using antibodies for phospho- ERK 1/2 (Thr 202/ Tyr 204) [Calbiochem, CA].

RESULTS

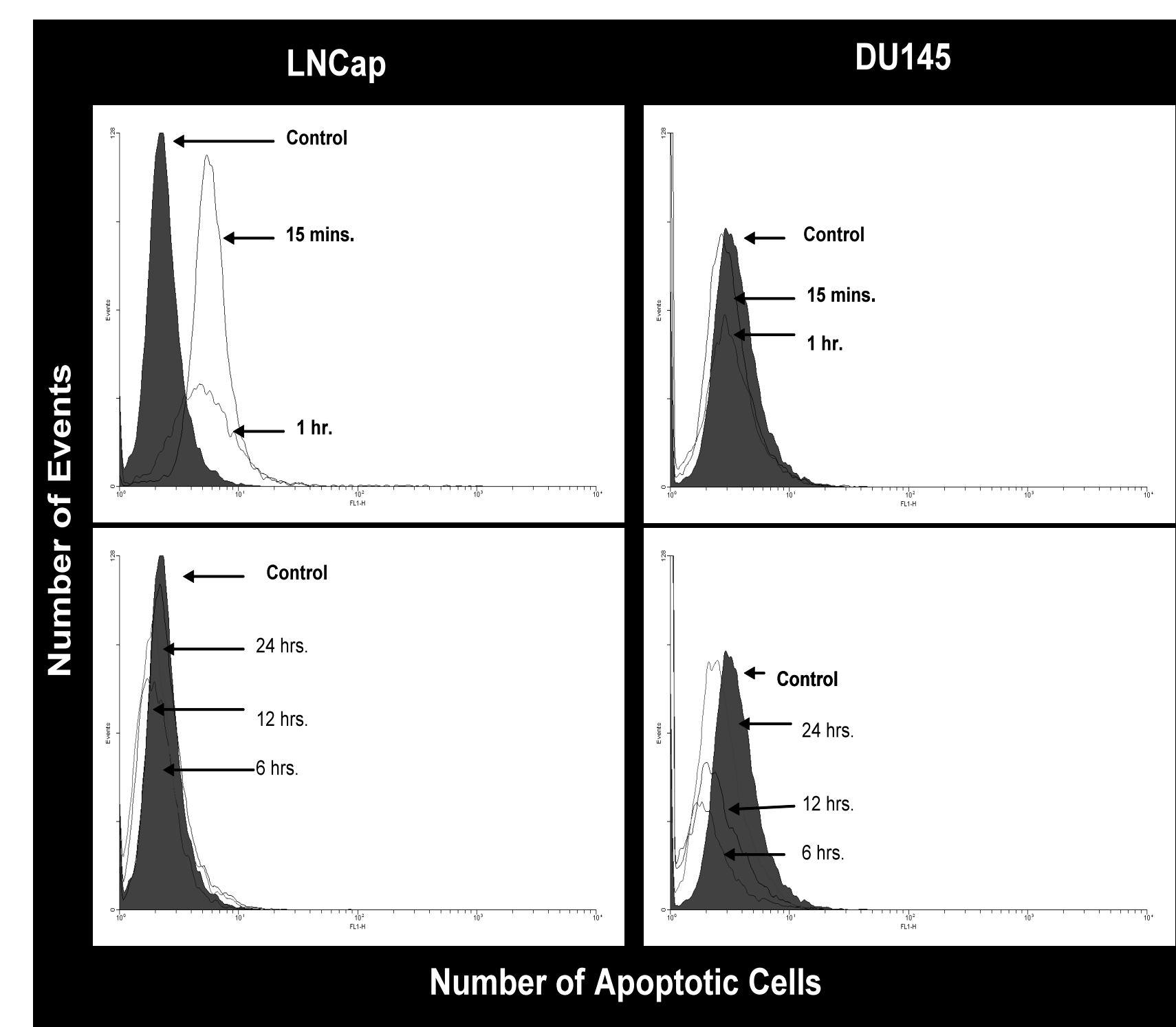


Figure 4- Alcohol inhibits prostate cancer cell death, apoptosis, at long time points of incubation
In LNCap cells, Alcohol at short time points seem to have tumor suppressive capabilities by inducing more cells undergoing apoptosis. In both prostate cancer cell lines studied, Alcohol promotes less cells undergoing apoptosis at 6 hours, 12 hours, and 24 hours.
After cells were incubated with 20mM Alcohol for 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours, apoptosis was examined by labeling of DNA breaks in apoptotic nuclei using Fluorescein FragEL DNA fragmentation Detection Kit [Calbiochem, CA] following flow cytometry analysis.

Demonstration of the Complexity of Cell Survival Signals.
Where does alcohol fit?
Further studies are aimed at better understanding alcohol affects on signal transduction pathways involved in cancer cell survival.

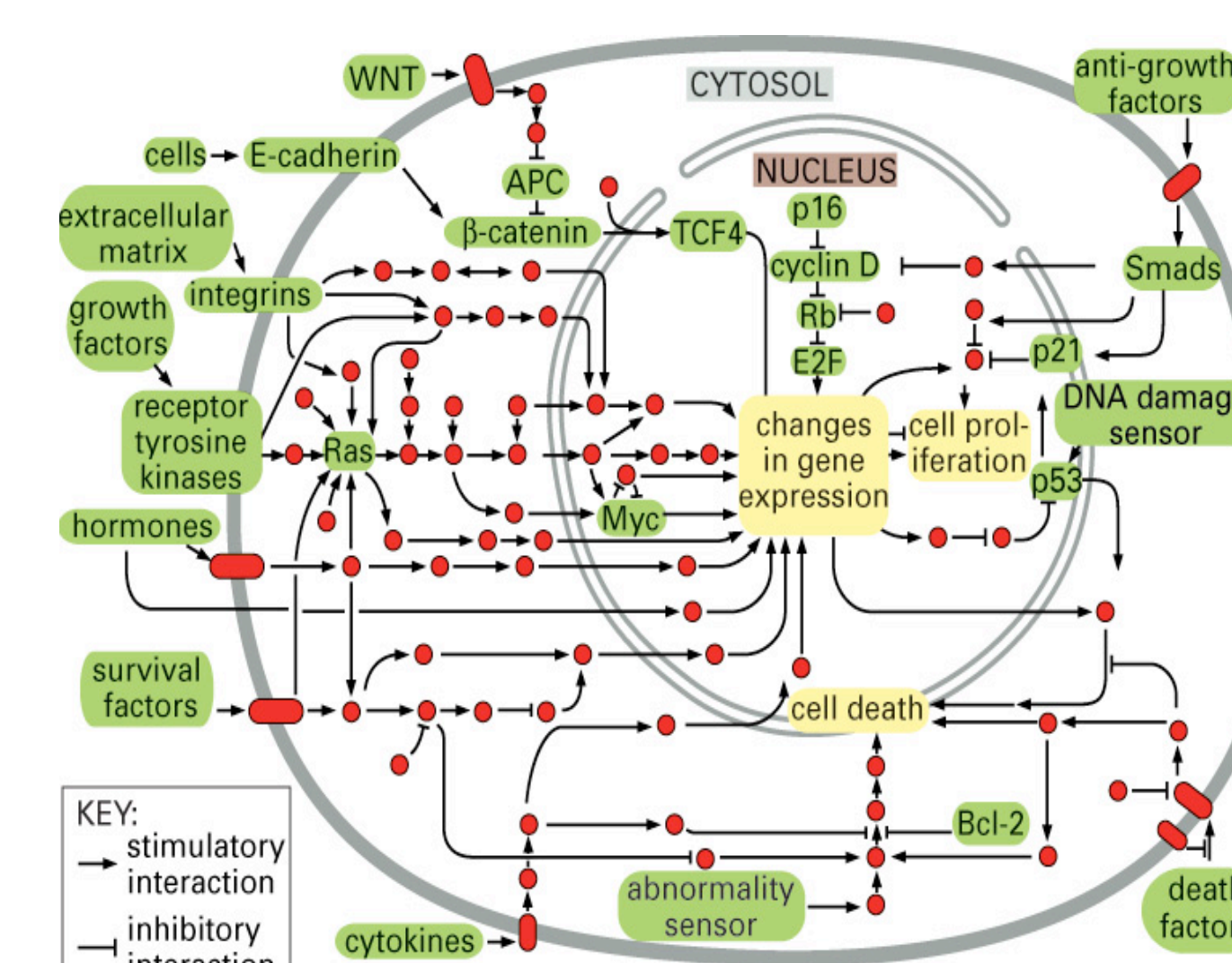


Figure 23-31. Molecular Biology of the Cell, 4th Edition.

All of which are also regulated at the genetic level.

Conclusions

- In prostate cancer cells, Alcohol activates the cell survival signal AKT at time points greater than or at 6 hours.
- In prostate cancer cells, Alcohol activates the cell survival signal ERK 1/2 at time points greater than or at 6 hours.
- The long time point at which Alcohol activates AKT and ERK 1/2 suggest that these signaling proteins are activated by changes in gene expression.
- Alcohol exposure reduces the number of prostate cancer cells undergoing programmed cell death, apoptosis, which further supports the idea that long-term exposure of Alcohol promotes an increase in cell survival of cancer cells.

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