



Dependence of Cell Cycle Checkpoint Proteins in HPV+ and HPV- Head and Neck Squamous Cell Carcinoma Cells Following 4NQO-Induced DNA Damage

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SHREVEPORT

ABSTRACT

Background: Head and neck squamous cell carcinoma (HNSCC) is known to be associated with certain environmental and lifestyle risk factors such as alcohol consumption, tobacco smoking, and exposure to UV light. In addition, particular strains of viruses including human papillomavirus (HPV) and Epstein-Barr virus are strong contributors to the development of HNSCC. Tobacco exposure is known to contribute to the oncogenesis of HNSCC through alteration of different molecular pathways, such as DNA damage repair (DDR) pathways, and mutation induction. Interestingly, in a previous study HPV positive (+) smokers and non-smokers had similar mutational profiles, which suggests that HPV could be decreasing mutation induction in smokers. 4-nitroquinoline-1-oxide (4NQO) is a mutagen and is often used as a treatment to mimic the effects of smoking. Previously, cell cycle and apoptotic signaling pathways played an important role in 4-nitroquinoline-1-oxide (4NQO)-induced tongue tumorigenesis. Our preliminary data has shown that 4NQO enhances activation of high-fidelity homologous recombination (HR) DNA repair proteins preferably in HPV+ HNSCC cells.

Hypothesis: In addition to increased DNA repair, HPV+ cells could also eliminate mutations through the activation of the DNA damage checkpoint to restrain the mitotic entry of damaged cells compared to HPV- cells. Therefore, we tested the effects of 4NQO on cell cycle phases and regulatory proteins in HNSCC cells in this study.

Materials & Methods: NOK E6.E7 +/-, UPCI-SCC40 (HPV-), UMSC-C104 and UM-SCC47 (HPV+) cells were treated with 4NQO (0.1 and 0.2 µg/ml) for 4, 6 and 24 hours. Expression of cell cycle regulatory proteins was analyzed by Western blotting. PI staining was done to check cell cycle phases followed by flow cytometry analysis.

Results: Western blot analyses revealed that 4NQO treatment at 6h resulted in significant increase in Chk1 and Chk2 phosphorylation in NOK E6.E7 and HPV+ HNSCC cells. p53 and p21 levels were also increased compared to HPV- cells, but cyclin D1 levels were not affected in any of the cell lines following 6h 4NQO treatment. Flow cytometry analysis showed that 4NQO treatment at 6h showed no change in cell cycle phases (data not shown). However, after 24h of 4NQO the G1 population increased in HPV- SCC40 HNSCC cells whereas the G2/M phase increased in HPV+ (SCC47 and SCC104) HNSCC cells whereas NOK E6.E7 +/- cells did not reveal any difference. We also observed an increase in pChk2 and decrease in cyclin D1 in NOK E6.E7 +/- and HPV+ HNSCC cells compared to HPV- cells after 24h 4NQO treatment.

BACKGROUND

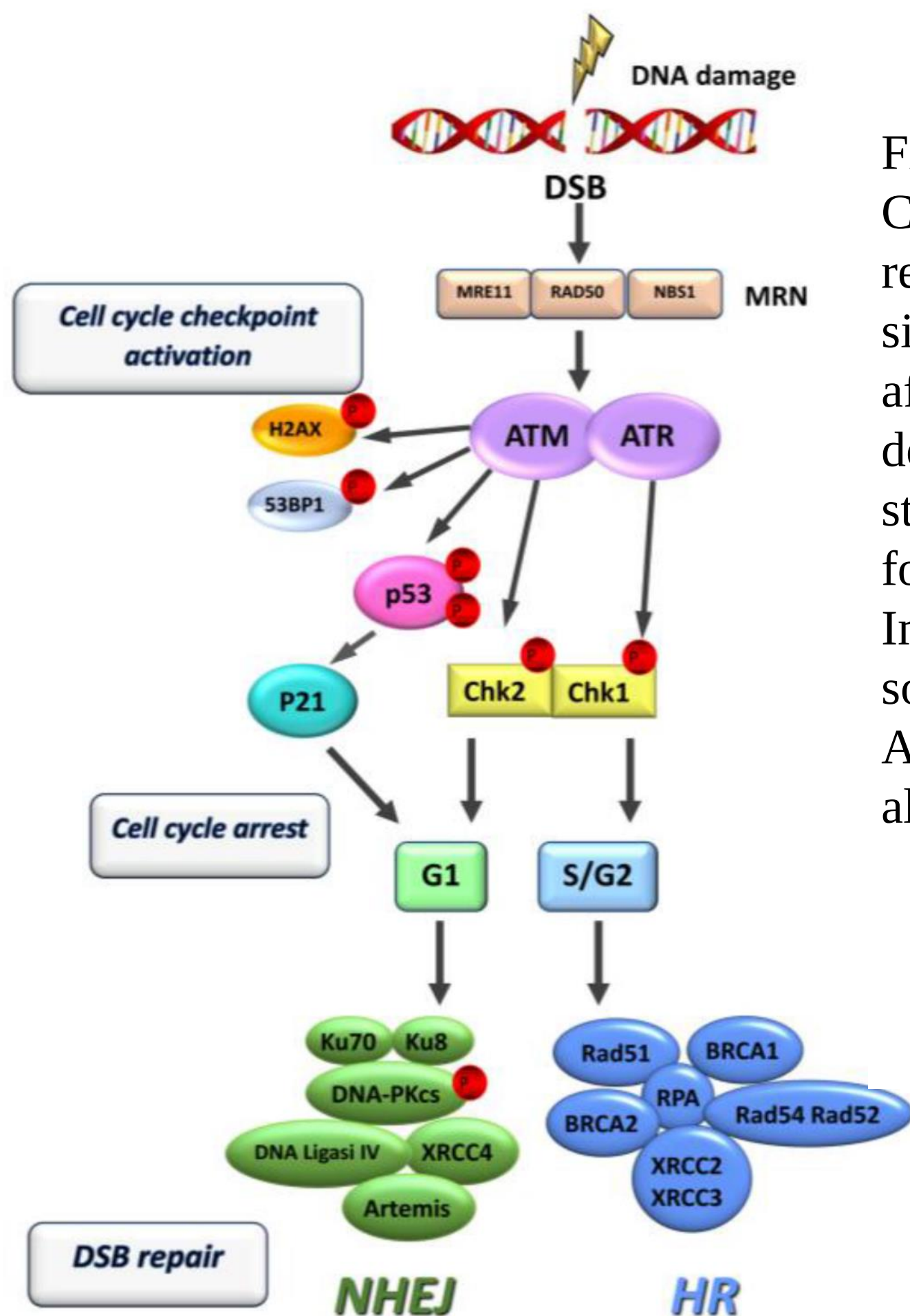


Figure 1- Checkpoint/repair signaling after DNA double strand break formation Image source- Annovazi et al, 2017

CONCLUSION

Taken together, our study suggests that 4NQO, a surrogate for cigarette smoking, differentially activates cell cycle checkpoint proteins which may help protect HPV+ HNSCC cells against smoking-induced mutations by allowing more time for HR repair. This could explain the better prognosis of HNSCC patients who are HPV+ and are smokers compared to HPV- smokers

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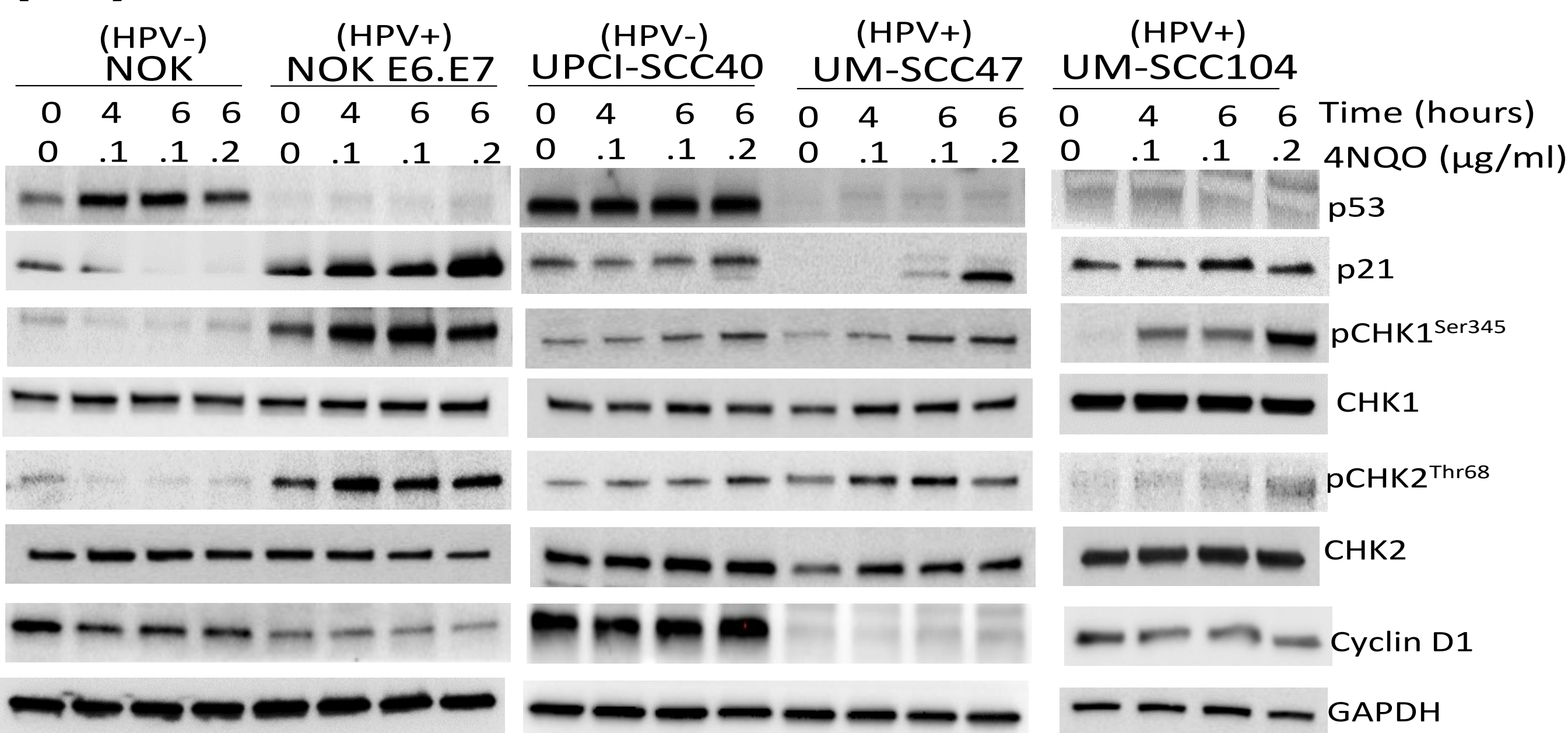
FUNDING SUPPORT

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RESULTS

4NQO induces more activation of cell cycle checkpoint proteins in HPV+ HNSCC cells compared to HPV- HNSCC cells

(1A)



(1B)

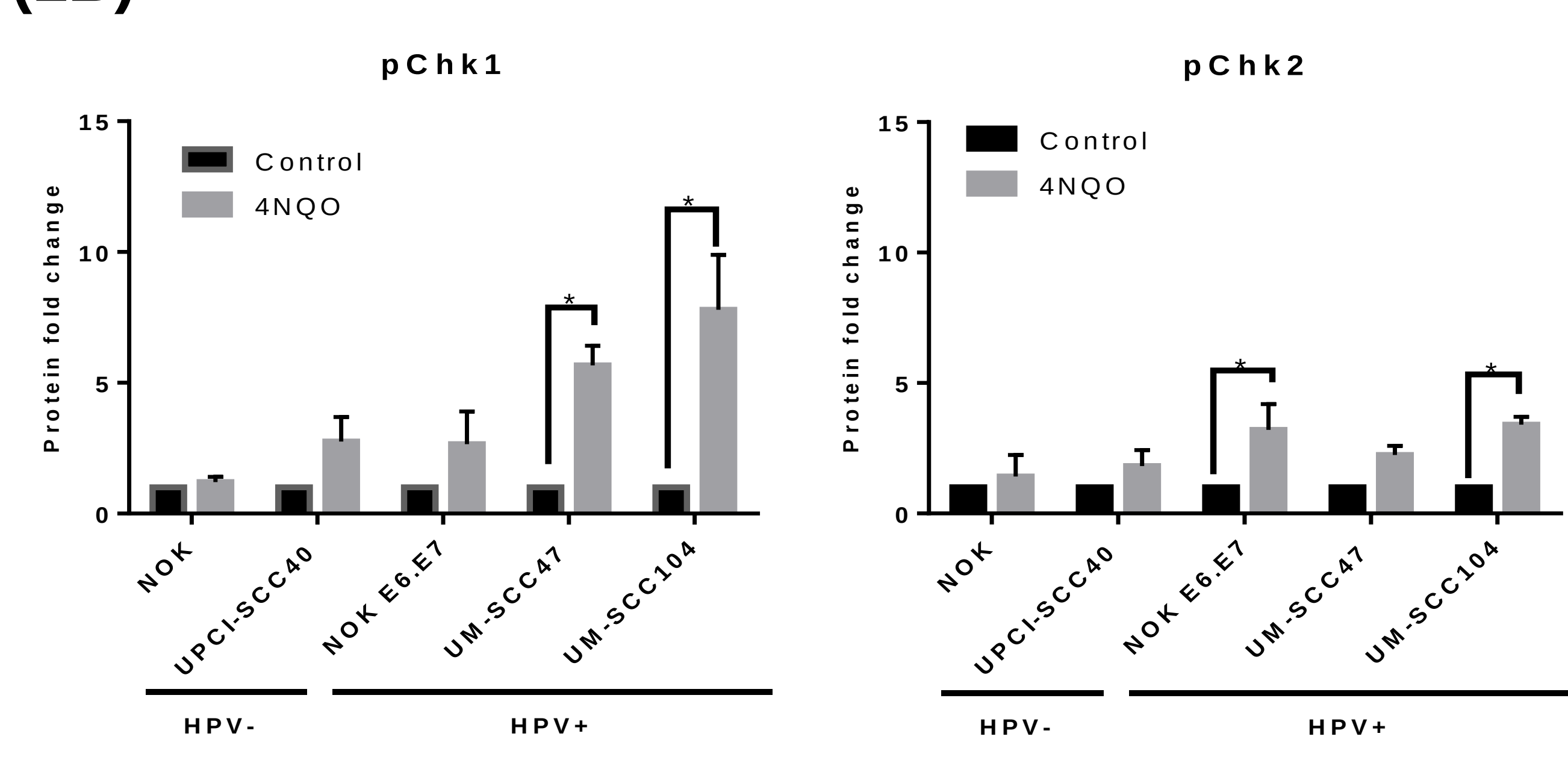


Figure 1- (A) Western blots showing activation/ expression of cell cycle checkpoint proteins in HPV- and HPV+ HNSCC cells after treatment with different concentrations (0-0.4µg/mL) of 4NQO for 4 and 6 hrs. **(B)** Graphs showing activation of pChk1 and pChk2 following 4NQO treatment in HPV- and HPV+ HNSCC cells. Error bars represent standard deviation.

4NQO increases G1 population in HPV- HNSCC cells and G2/M population in HPV+ HNSCC cells

(2)

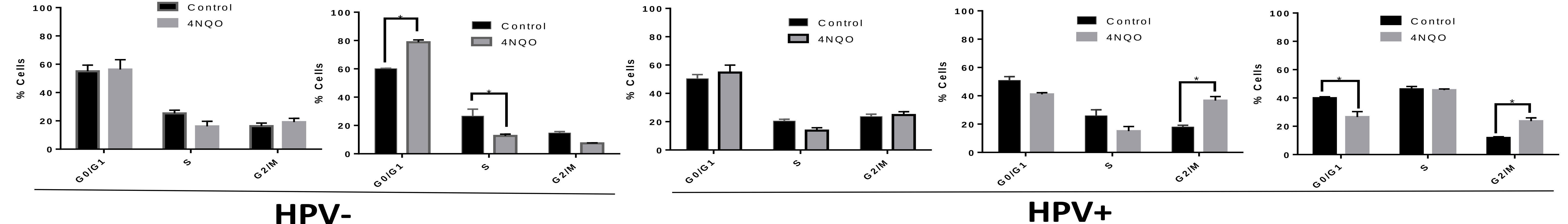


Figure 2- Graphs showing the effect of 4NQO on cell cycle phases in response to control and 4NQO treatment after 24 hours in HPV- and HPV+ HNSCC cells. Error bars represent standard deviation.

(3)

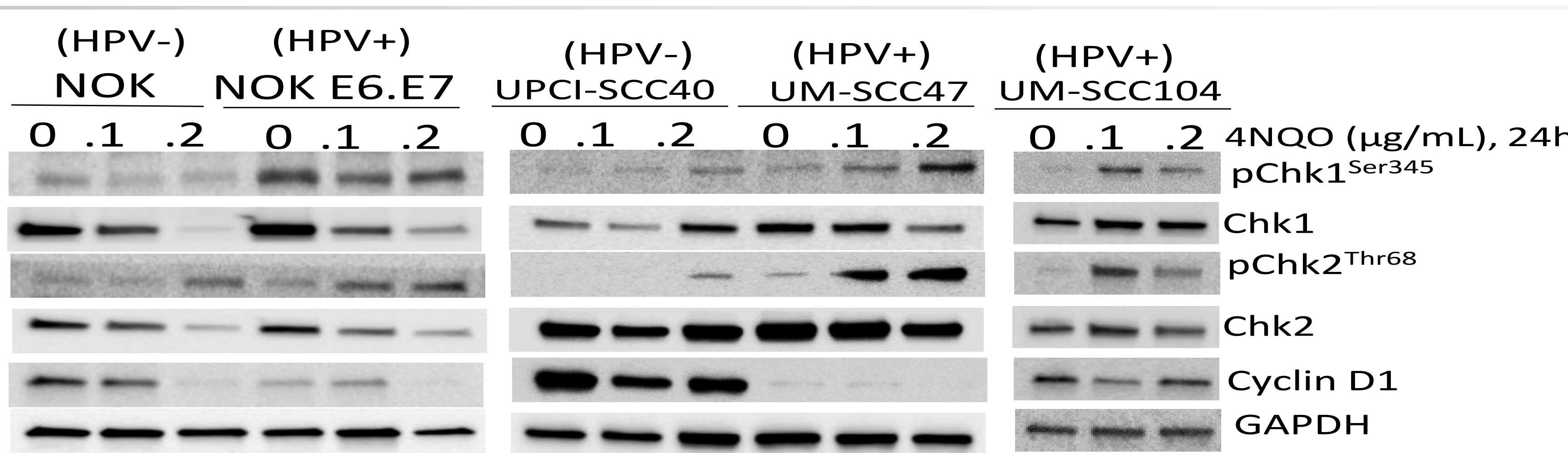


Figure 3- Western blots showing activation/ expression of cell cycle checkpoint proteins after treatment with different concentrations (0-0.4µg/mL) of 4NQO for 24 hrs in HPV- and HPV+ HNSCC cells .

Longer treatment of 4NQO induces more activation of Chk2 in HPV+ HNSCC cells compared to HPV- HNSCC cells