

Background

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy in the world that develops in mucous membranes of mouth, nose, and throat. Since with current treatments the 5-year survival rate for patients at higher stages is less than 50% there is a need for novel treatments. HNSCC is characterized by dysregulated cell growth rate and aberrant protein synthesis leading to accumulation of misfolded proteins that triggers Unfolded Protein Response (UPR). To address a new treatment, we proposed Acetyl-isogamabogic acid (AIGA) as a compound that could enforce terminal (apoptotic) UPR in HNSCC. Further cell proliferation assays, gene expression, protein immunoblot profiles, DNA fragmentation assay, and fluorescence activated cell sorting (FACS) demonstrated activation of UPR and apoptosis in HNSCC cell lines.

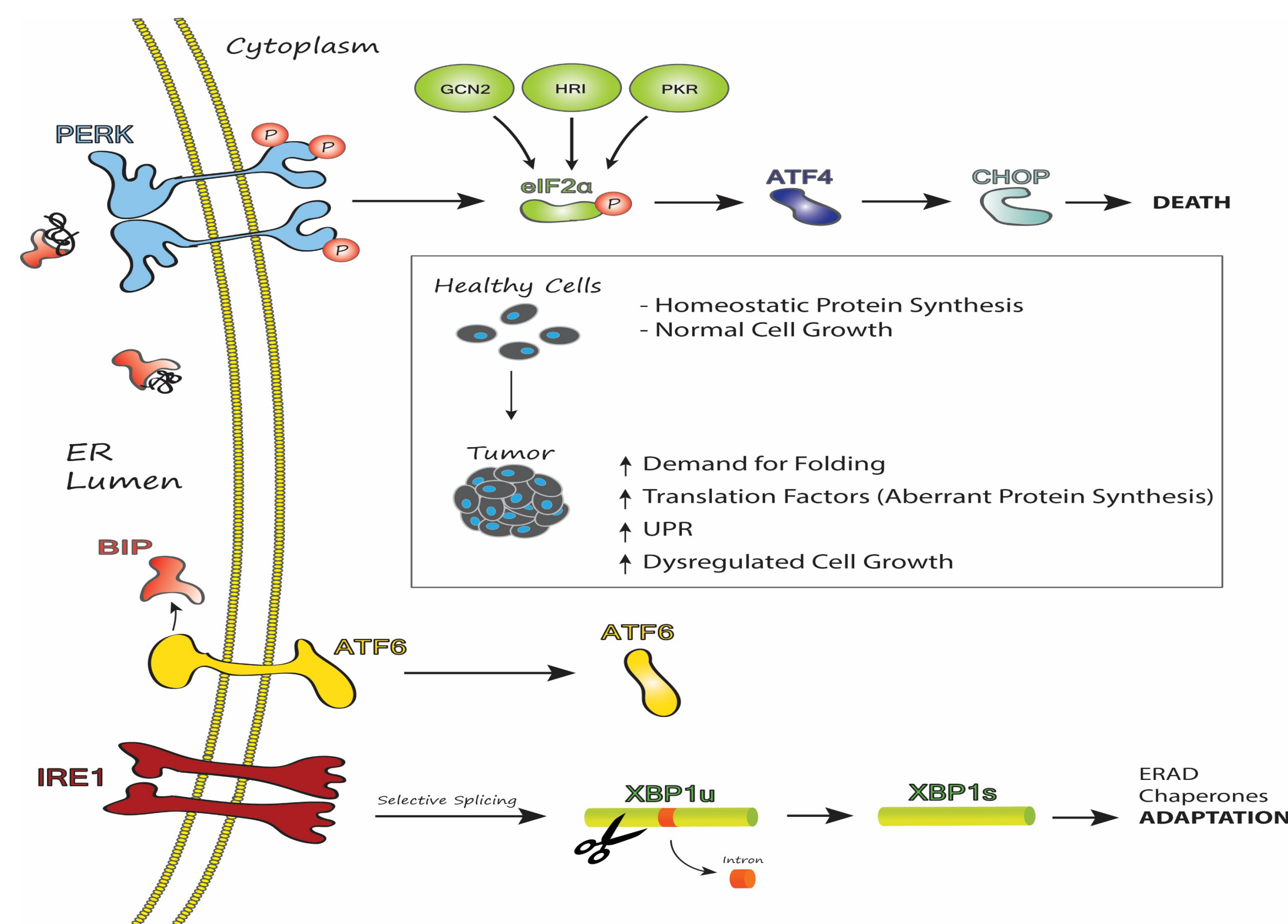


Figure 1. General model of ER stress and UPR signaling

Results

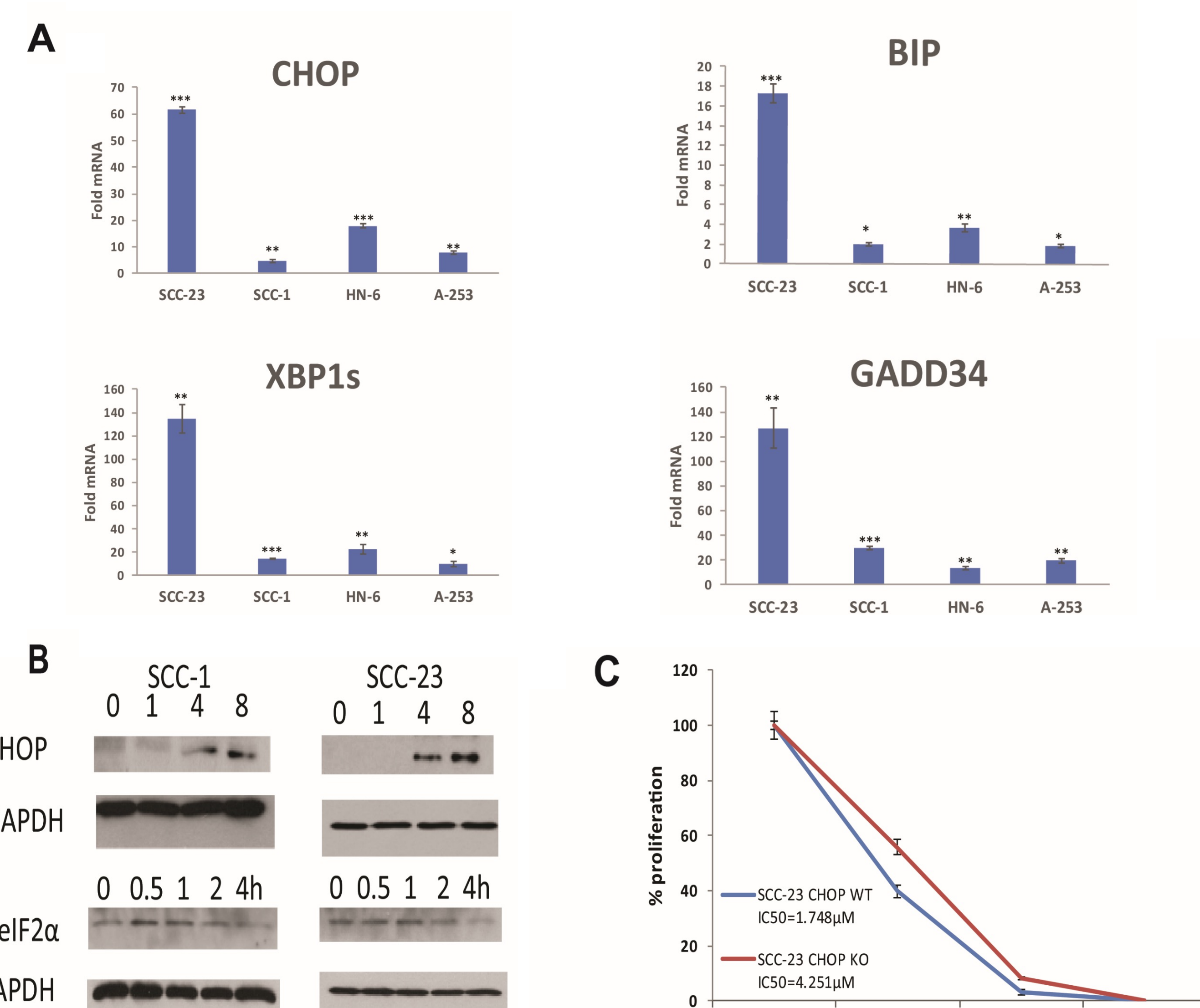


Figure 2. AIGA induced ER stress and the UPR in HNSCC. HNSCC cells were treated with 5 μM AIGA for 6hr and cell lysates were interrogated for UPR gene (A) or protein expression (B). Concentration response assays revealed that CHOP-null SCC-23 cells were less sensitive than WT cells to the antiproliferative effects of AIGA (C).

Results

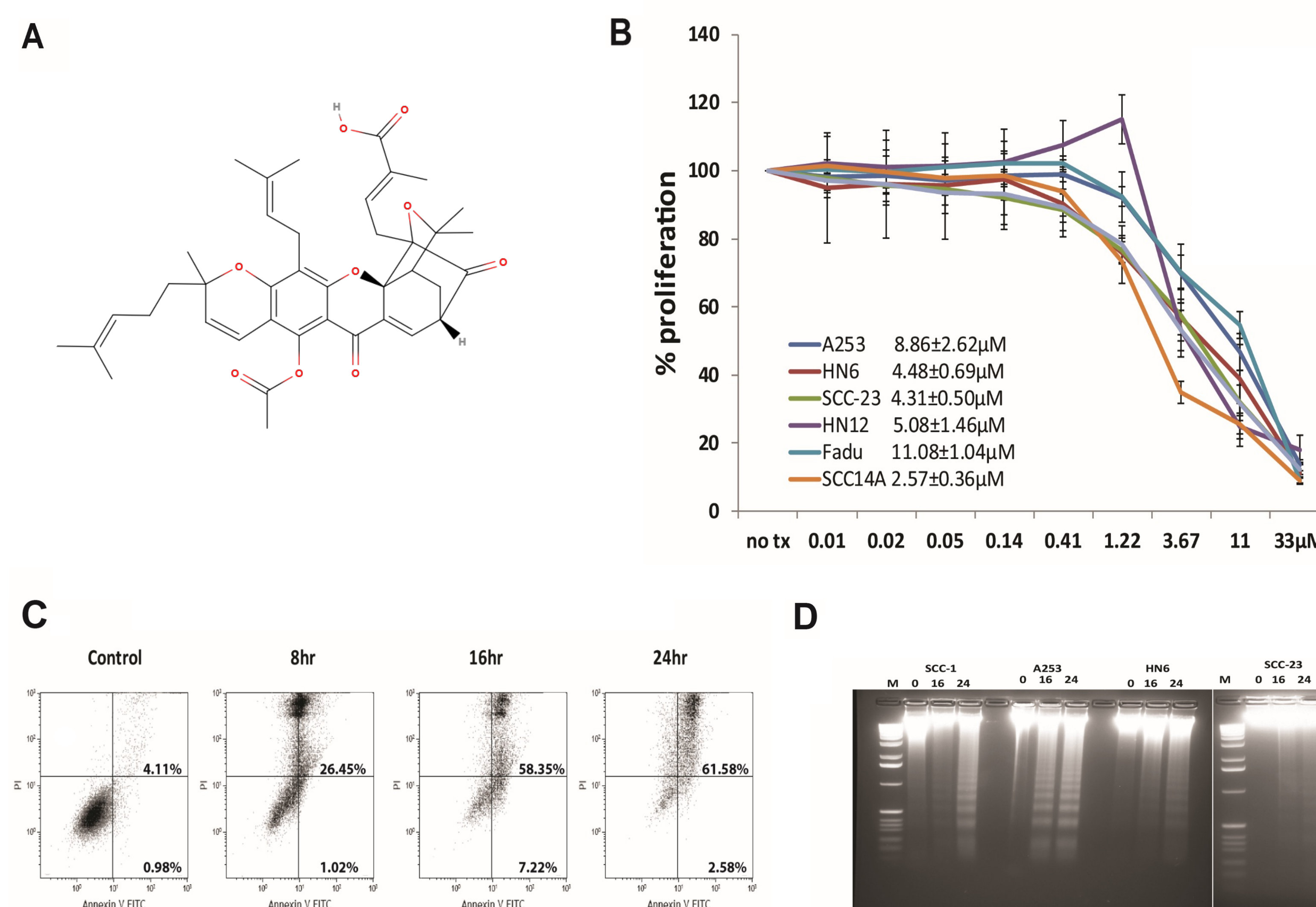


Figure 3. AIGA reduced proliferation and induced apoptosis. AIGA (A) was used to perform concentration response proliferation assays (MTT) after 24 h (B). FACS analysis for markers of apoptosis demonstrated increased annexin V and propidium iodide staining over time, as indicated (C). DNA fragmentation assays revealed that AIGA could induce apoptosis in HNSCC (D).

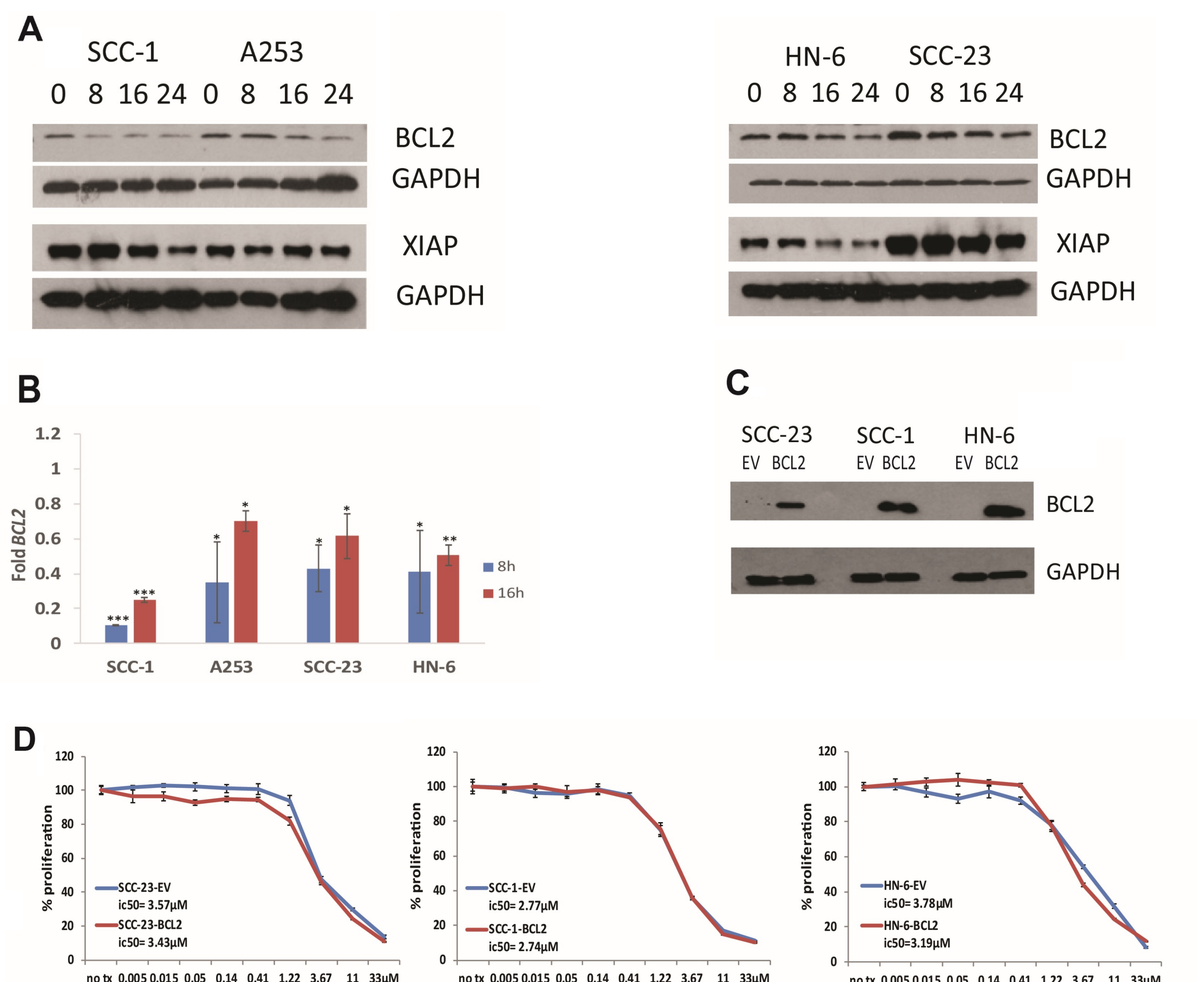


Figure 4. AIGA-induced apoptosis is preceded by decreased expression of pro-survival proteins. HNSCC cells were treated with 5 μM AIGA as indicated. Analysis of whole cell lysates revealed decreased expression of BCL2 and XIAP (A) *BCL2* (B). Overexpression of BCL2 (C) could not protect HNSCC cells from the antiproliferative effects of AIGA (D).

Results

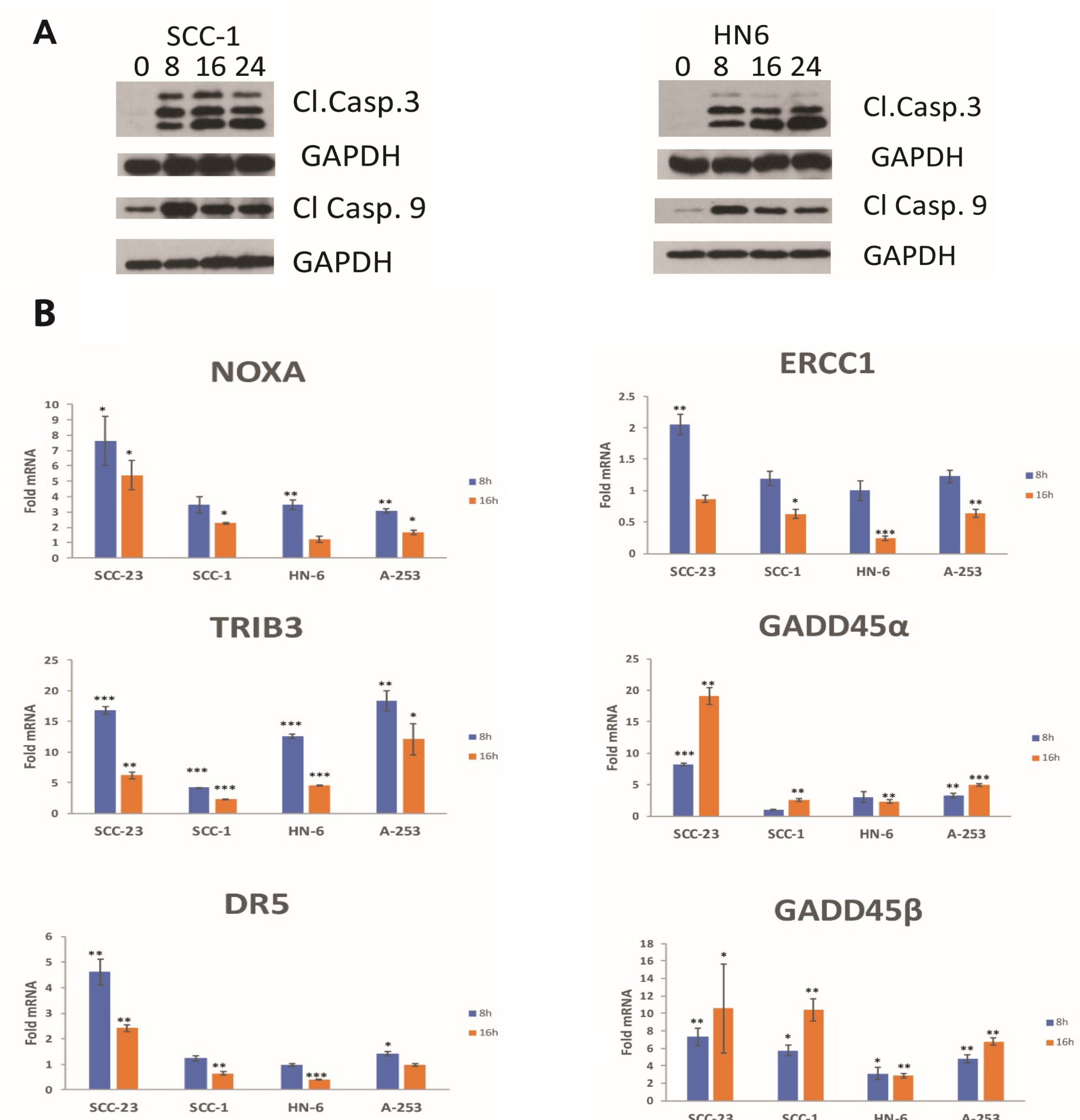


Figure 5. AIGA increased apoptosis-related biomarkers in HNSCC. 5 μM AIGA cleaved caspase proteins (A) and increased apoptosis-related gene transcripts, as indicated (B).

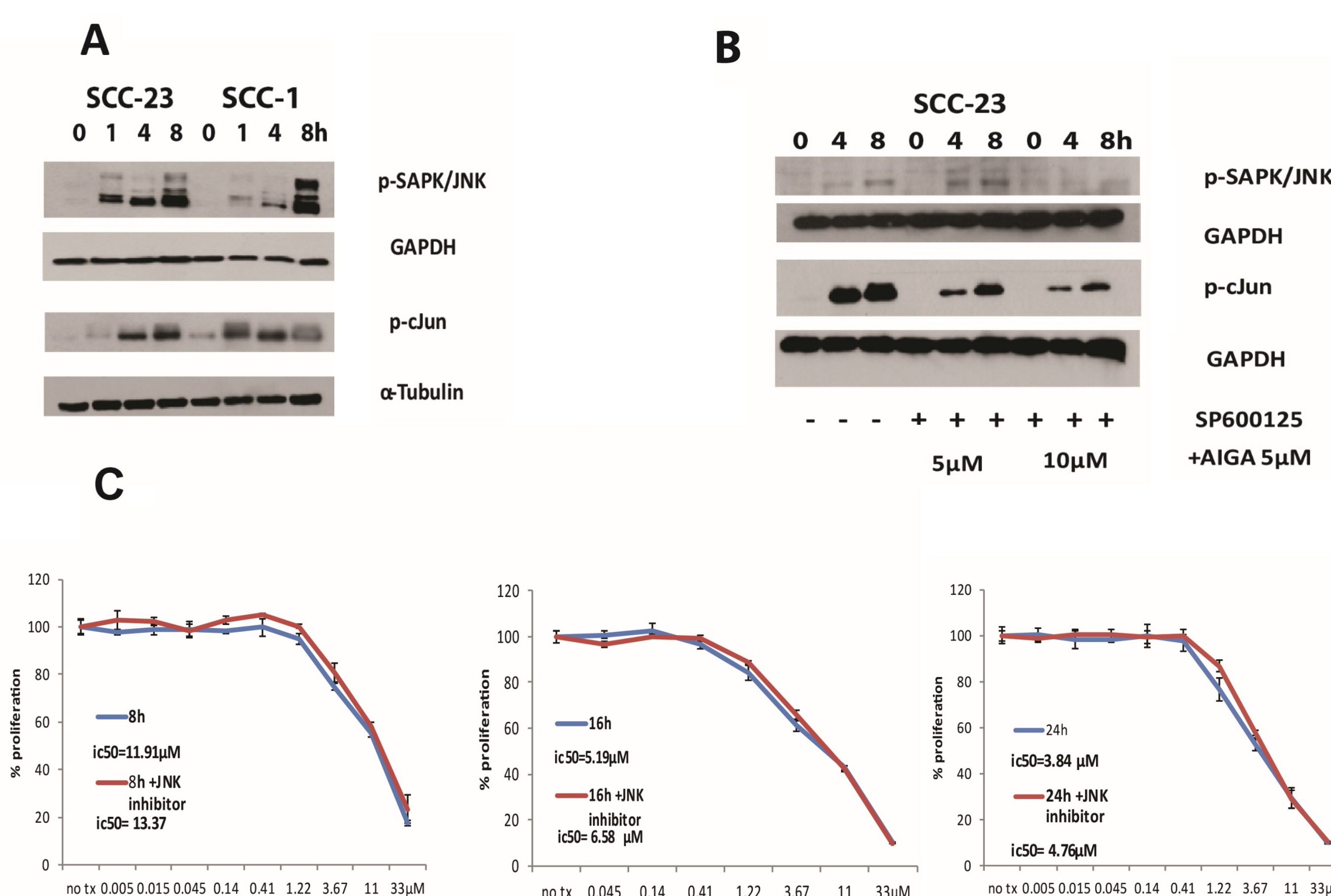


Figure 6. AIGA activated JNK signaling in HNSCC. 5 μM AIGA induced phosphorylation of JNK and c-Jun (A). The JNK inhibitor SB600125 reduced P-JNK and C-Jun (B) but did not protect SCC-23 cells from AIGA over time (C).

Conclusions

1. AIGA induced ER stress and an UPR in HNSCC.
2. Unmitigated ER stress/UPR induced apoptosis and reduced HNSCC proliferation.
3. CHOP-null SCC-23 cells were significantly less sensitive to AIGA.
4. Neither BCL2 overexpression, nor JNK inhibition rescued HNSCC cells from AIGA
5. Further preclinical studies, currently underway, are focused to elucidate the therapeutic potential of AIGA for HNSCC