

Effect of opioid receptor activation and blockage on the progression and response to treatment of head and neck squamous cell carcinoma

Lirit Levi*, Hagit Shoffel-Havakuk*, Elad Hikri, Avraham Dayan, Aron Popovtzer, Gideon Bachar, Aviram Mizrahi

Department of Otorhinolaryngology - Head and Neck Surgery, Rabin Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel



Background: Patients with Head and neck Squamous Cell Carcinoma (HNSCC) have the highest prevalence of pain, mostly controlled with opioids. Recent studies on other malignancies suggest that opioids may influence cancer progression. Moreover, opioid abuse appears to be a risk factor for specific types of HNSCC. Mu Opioid Receptors (MOR), were found to be upregulated in several types of human cancer cells including HNSCC, however the effects of their activation or blockage remains unclear.

Objectives: This study aims to investigate the mechanism of MOR activation and blockage in pre-clinical models of HNSCC, with relation to disease progression, response to therapy and activation of the PI3K/AKT/mTOR signaling axis, a frequently activated signaling route in HNSCC.

Methods: Expression and quantification of MOR-1 was assessed in Seven different HNSCC cell lines using western blot (WB). XTT assays of cell proliferation were performed on 4 selected HNSCC cell lines. These experiments were repeated with the following conditions: exposure to opiate receptor agonist (Morphine), antagonist (Naloxone), alone and combined with Cisplatin. Finally, the effects of Morphine and Naloxone on the PI3K/AKT/mTOR signaling axis were analyzed using WB.

Results

MOR-1 expression in HNSCC cell lines

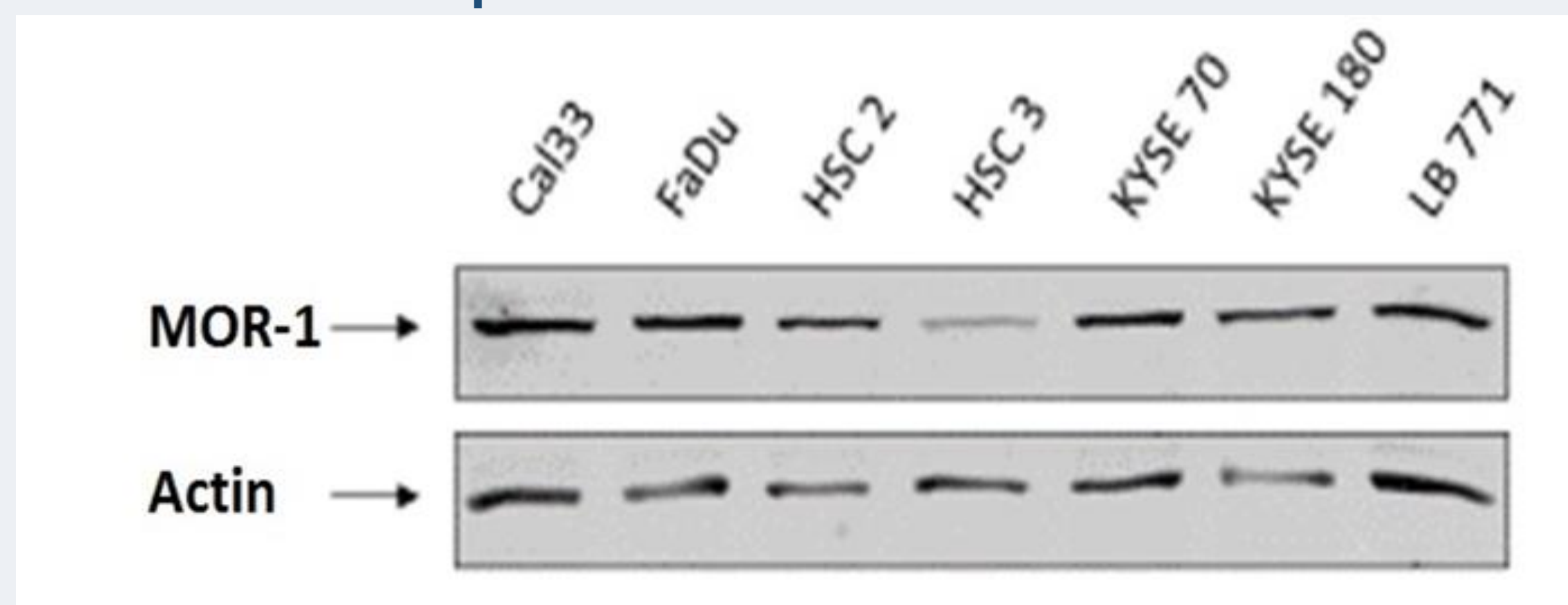


Figure 1. Western blot analysis for MOR-1 expression in seven HNSCC cell lines. All seven cell lines demonstrated MOR-1 expression. Cal33, FadU and HSC-2 had high expression of MOR-1, while HSC-3 showed the modest expression. Therefore, we focused on those four cell lines.

Morphine increased cell proliferation in four cell lines

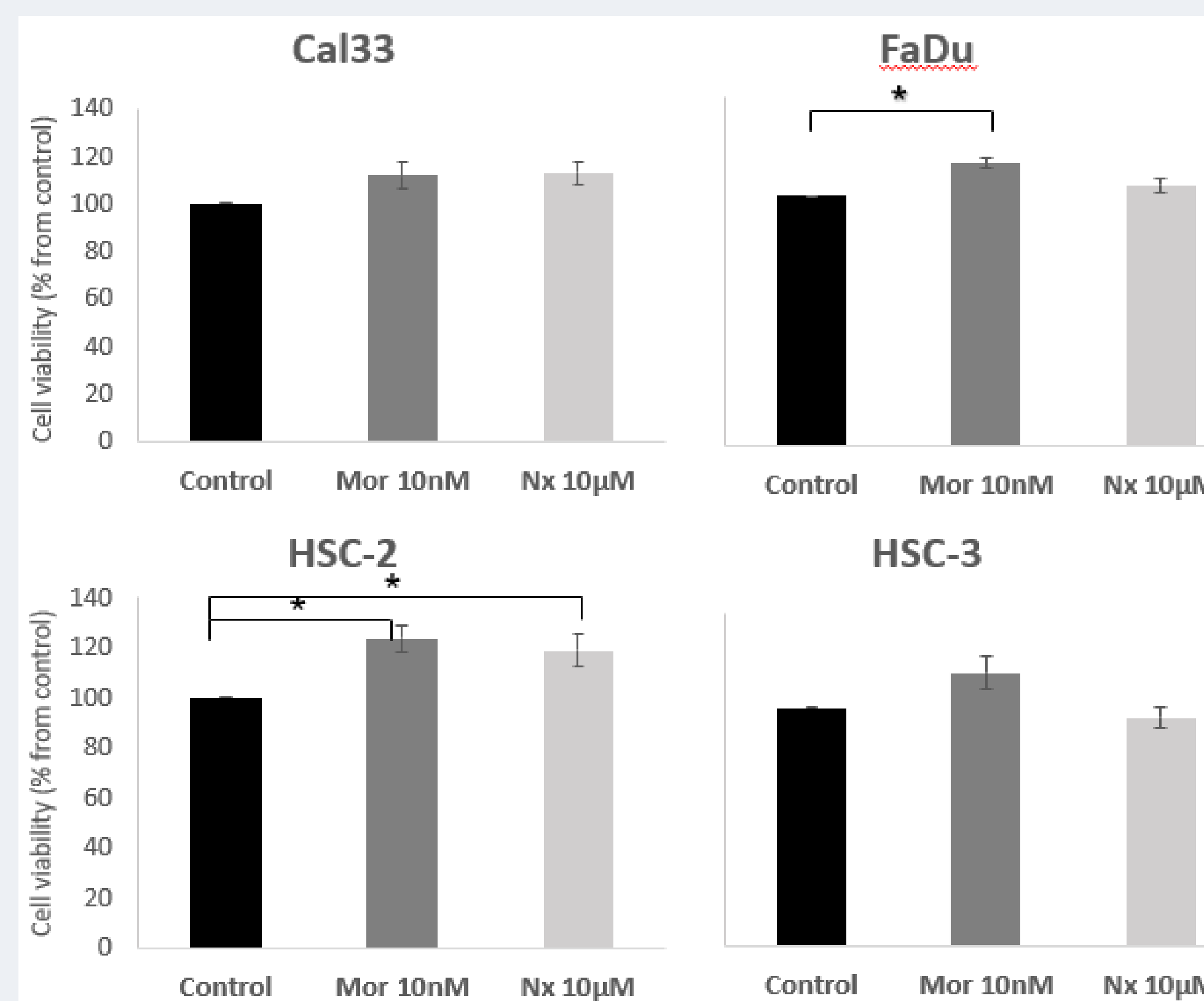


Figure 2. XTT cell viability assay of four cell lines incubated with Morphine or Naloxone. Morphine induced an increase in cell proliferation in all four cell lines.

*P-value<0.05

Opioid receptor agonist and antagonist effect on response to Cisplatin

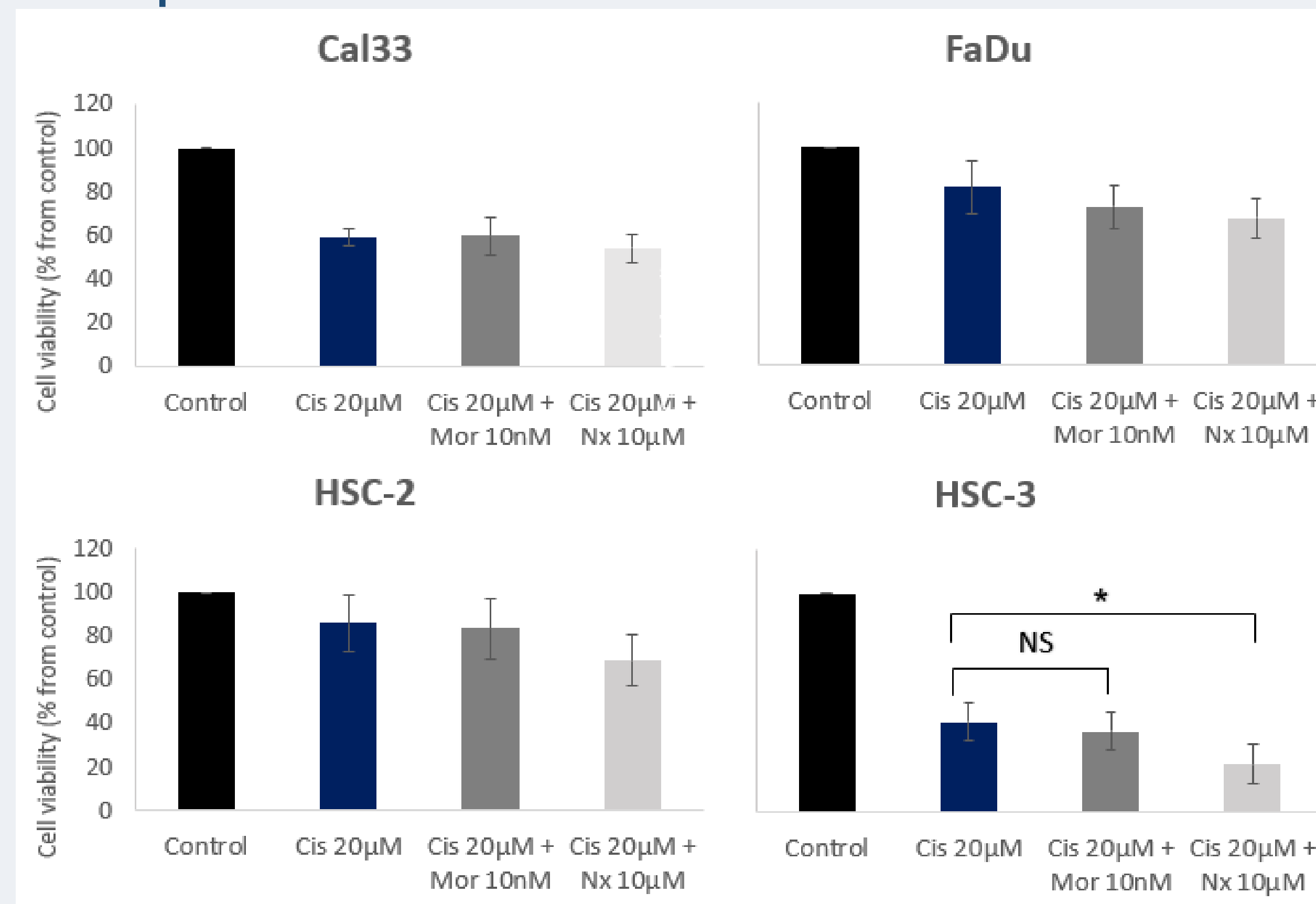


Figure 3. XTT cell viability assay of four cell lines incubated with Cisplatin, Morphine or Naloxone. The addition of Naloxone to Cisplatin resulted in a decline in cell viability in all four cell lines.

Morphine induced activation of key proteins in the PI3K/AKT/mTOR signaling pathway

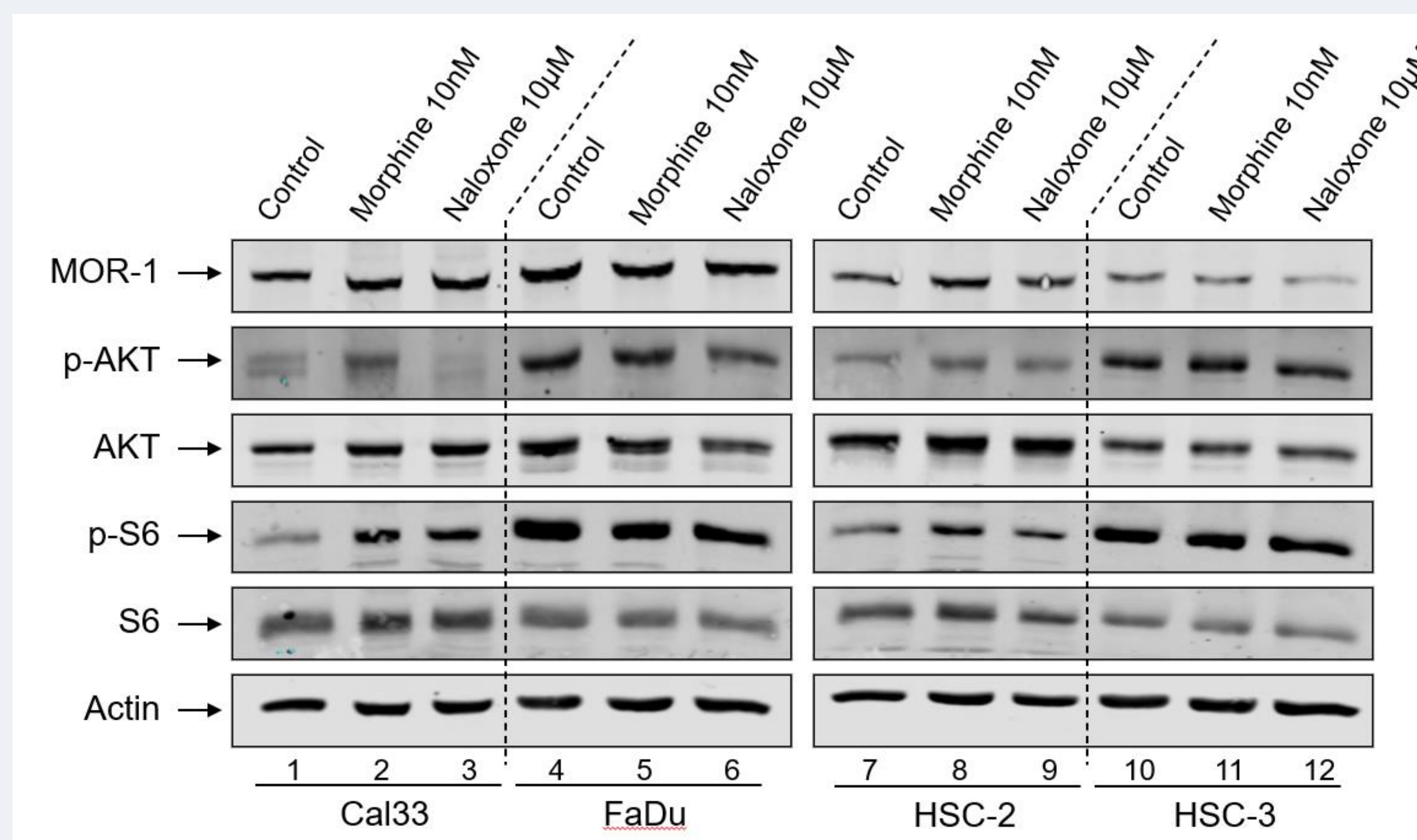
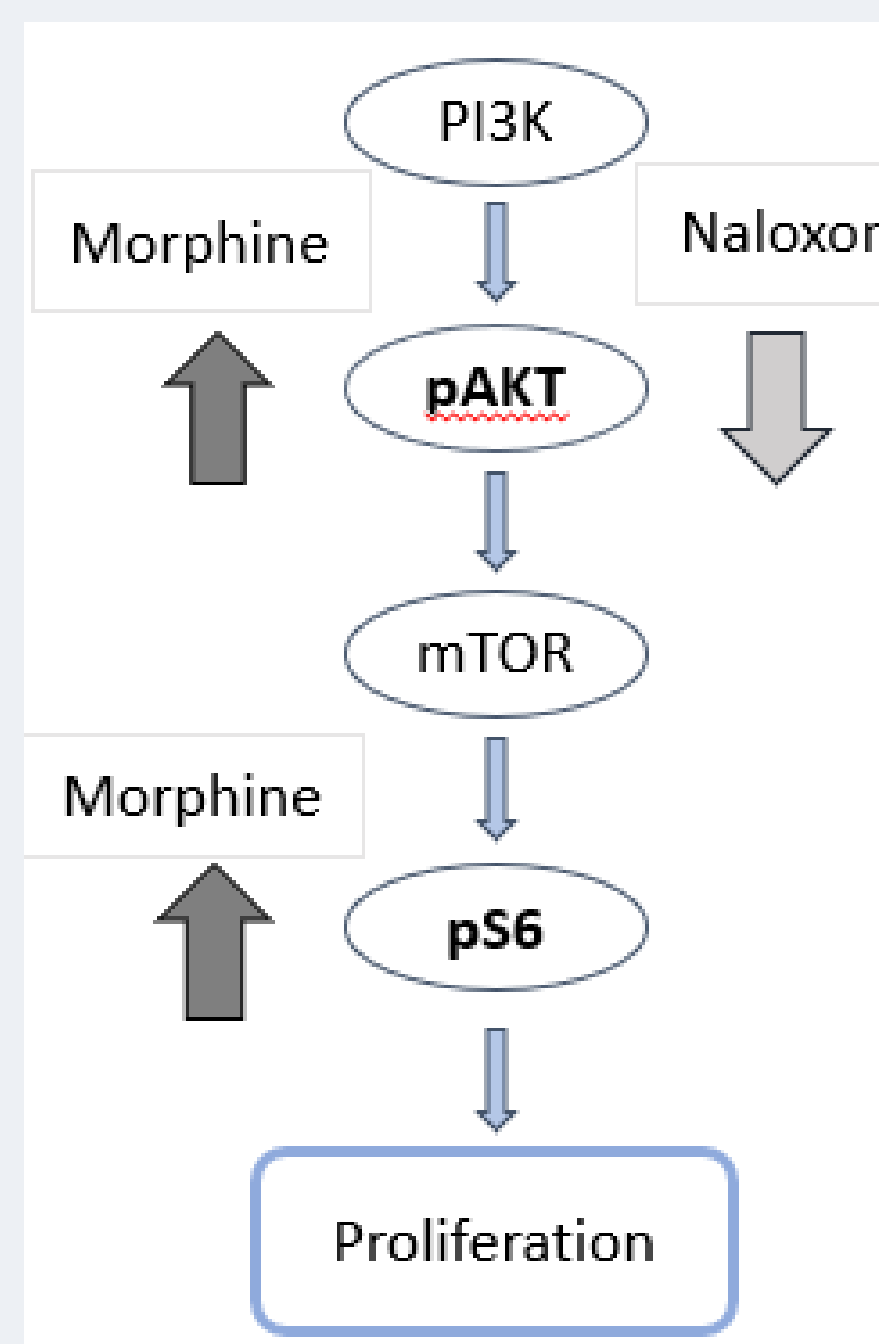


Figure 4. Western blot analysis of four cell lines incubated with Morphine or Naloxone, for the expression of AKT and phosphor AKT (p-AKT) and S6 and phosphor S6 (p-S6), key proteins in the PI3K/AKT/mTOR axis. Morphine increased levels of p-AKT in all cell lines, significantly in HSC-3 (p=0.039). Morphine increased levels of p-S6 in two cell lines. This indicates activation of the PI3K/AKT/mTOR axis. Naloxone attenuated levels of p-AKT in two cell lines, indicating inhibition of the axis.



conclusion

The findings of this preclinical study suggest that opioids may increase HNSCC cell proliferation via activation of the PI3K/Akt/mTOR signaling pathway. Moreover, MOR blockage may chemo-sensitize HNSCC to cisplatin.