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Benzethonium chloride activates ER stress and reduces proliferation in HNSCC

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy in the world, accounting for 650,000 new cancer diagnoses each year with 350,000 deaths [1]. Despite the high mortality rate and increased public interest in the United States, NIH support for head and neck cancer research has been decreasing and progress in treatments has accordingly lagged egregiously behind more generously funded malignancies [2–4]. Management of HNSCC over the past 20 years has moved towards an organ preservation approach with most patients being offered a combination of chemotherapy and radiation treatment. Recent advances, including the development of immune modulators, are promising but have not yet significantly impacted disease-free survival.

Studies conducted by our group have previously demonstrated that activating a terminal unfolded protein response (UPR) was a critical component of the mechanism by which bortezomib (PS-341, Velcade) induced apoptosis in a diverse panel of HNSCC cell lines [5,6]. The current work describes a novel cell-based high throughput screen (HTS), genetic counterscreen, and a suite of orthogonal (secondary) assays honed to identify small molecules from large compound libraries that activate the UPR and reduce HNSCC proliferation in a CHOP-dependent fashion.

The UPR is a coordinated mechanism, which allows a cell to respond to stresses that disturb normal protein folding in the lumen of the endoplasmic reticulum (ER). The UPR is mediated through the activation of PERK, ATF6 and IRE1α, three ER transmembrane proteins that are held in an inactive state during homeostasis. When the level of peptides that needs to be folded outpaces the ability of the ER to process them, the chaperone glucose regulated protein 78 (GRP78/BiP) disassociates from PERK and ATF6 resulting in their activation [7]. Alternatively, the binding of unfolded peptides to a luminal domain of IRE1α leads to its activation [8]. Activation of these three sensors simultaneously stimulates two genetically distinct pathways that attempt to resolve the encumbrance of misfolded proteins in the ER or lead to apoptotic dismantling of the cell (Fig. 1) [9,10]. IRE1α activates an exoribonuclease that results in an alternative splicing pattern for *XBP1* mRNA in the cytoplasm. XBP1 translation leads to the production of proteins (e.g., chaperones and foldases) that are critical to restore operative protein folding, and for ER-associated degradation (ERAD) of misfolded

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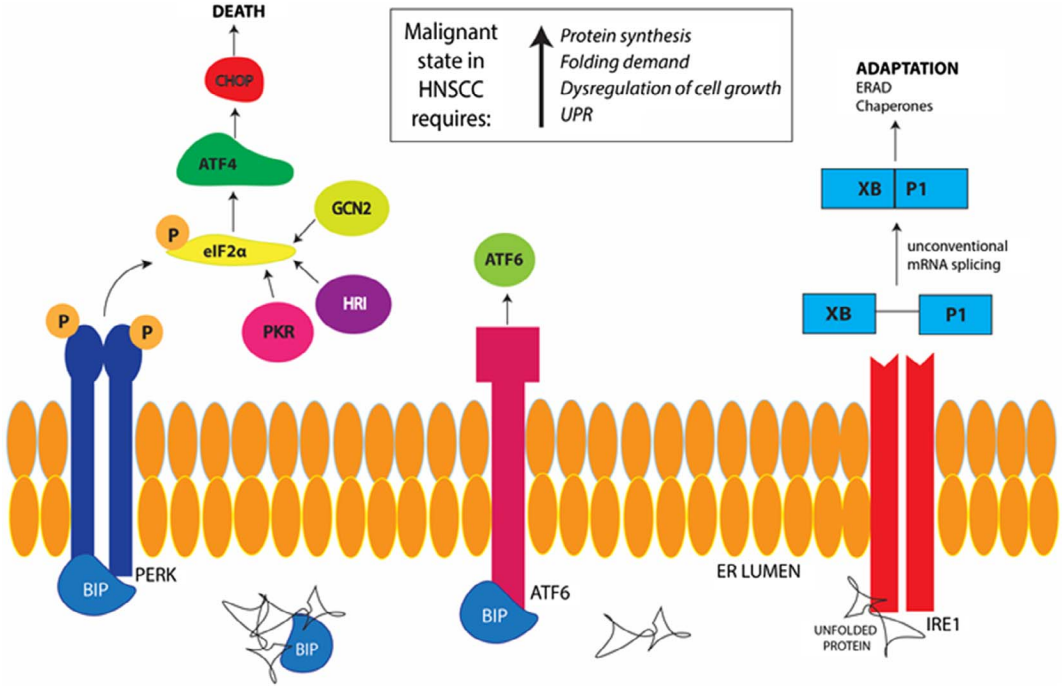

Fig. 1. General diagram of the unfolded protein response (UPR) and summary of aberrant UPR activation in HNSCC (inset).

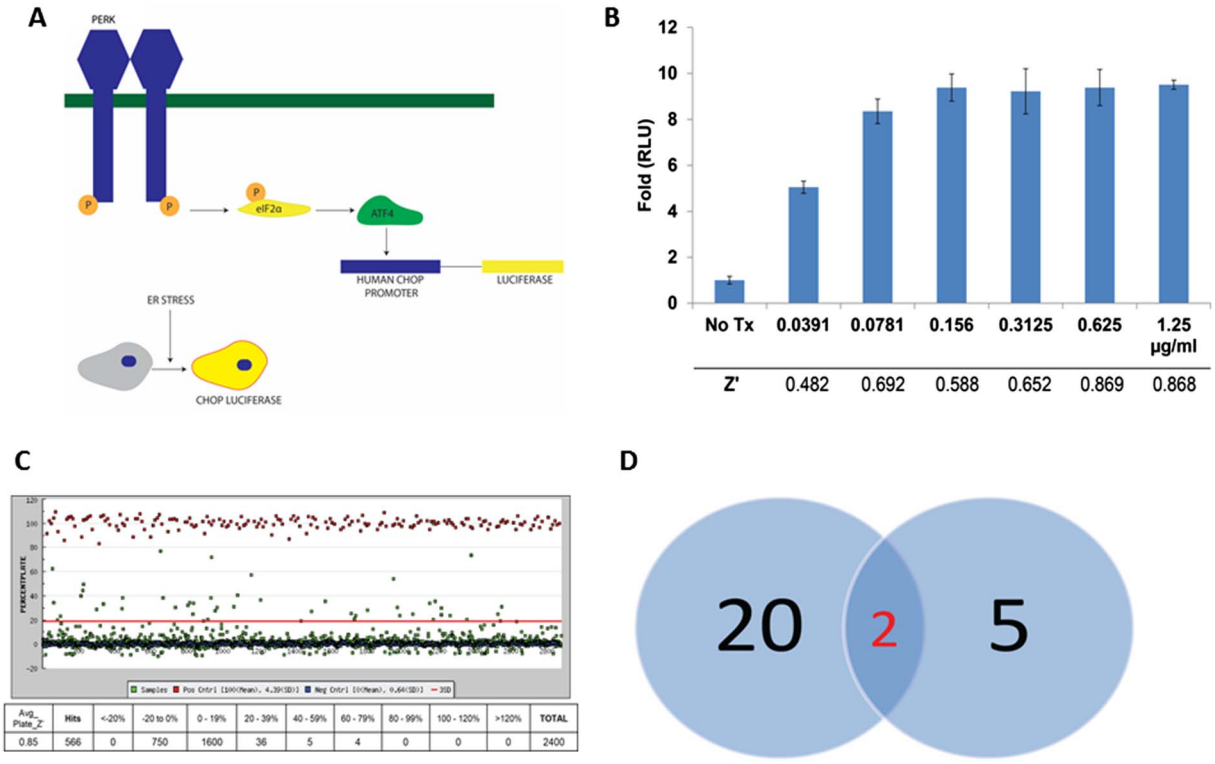

Fig. 2. SCC23-CHOP-luc cells identify unique hits. (A) Schematic representation of the human CHOP-luciferase reporter construct used to generate the HNSCC reporter cell line. (B) SCC23-CHOP-luc cells treated with increasing concentrations of tunicamycin (Tm) provide a statistically robust HTS assay in a 96 well format. (C) HTS campaign view of Microsource Spectrum Collection HTS library screened on SCC23-CHOP-luc; data reported as % luciferase (RLU). DMSO treated wells (blue baseline); Tm treated wells (red line = 100%); green dots are results from individual compounds (~2400). (D) Venn diagram comparison of hits using the rodent Chop-luc reporter in CHO-K1 cells (left) versus the human CHOP-luc reporter stably transfected in a relevant (HNSCC) cell line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

peptides by the 26S proteasome [11,12]. Autophosphorylation of PERK leads to the phosphorylation of the eukaryotic initiation factor 2-alpha (eIF2α) at Ser 51, which inhibits the initial step of polypeptide synthesis (i.e., the catalysis of MET to new peptides), resulting in a general pause in translation that serves to provide the ER an opportunity to recover [13]. Additionally, phosphorylation of eIF2α by PERK leads to the transcription and translation of ATF4 and CHOP, transcription factors that form homodimers with other C/EBP transcription factors, resulting in increased production of the pro-apoptotic proteins NOXA, DR5 and TRB3, and inhibition of the anti-apoptotic factor BCL2