Mechanism Underlying Downregulation of CBP & MLL1 following E7386-Mediated Inhibition of β-catenin-CBP Interaction

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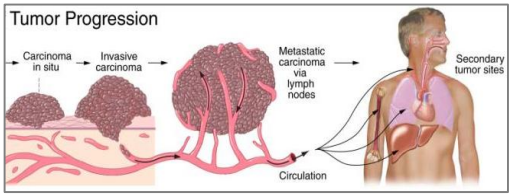
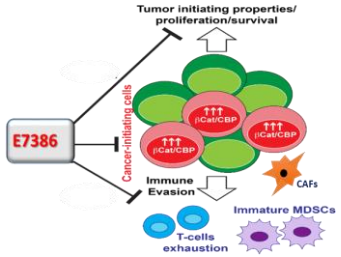
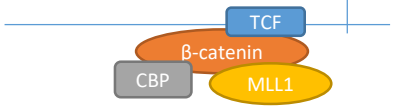
Head and neck cancer is a devastating disease with only ~60% overall 5-YSR. HNSCC cases are mainly present as HPV(-) OSCC with limited available treatments. Our previous studies showed that in OSCC the nuclear branch of the Wnt/β-catenin signaling pathway, the β-catenin/CBP axis, is an epigenetic regulator of cell survival, maintenance, and expansion of CSCs, EMT subpopulations. β-catenin/CBP signaling is an epigenetic driver of an active chromatin structure through H3K27 acetylation and H3K4 trimethylation (via MLL1). Our recent studies demonstrated that treatment of OSCC cells with a small molecule inhibitor of β-catenin-CBP interaction, E7386, dramatically reduced H3K4me3 occupancy at the transcription sites of numerous CSC genes. E7386 treatment did not affect steady-state levels of nuclear β-catenin, but it dramatically reduced the abundance of CBP and MLL. To identify the mechanism underlying destabilization of CBP and MLL1 by E7386 we tested the involvement of the ubiquitin-proteasome pathway. We first assessed whether the ubiquitin-activating enzyme, E1, was involved in the degradation of CBP and MLL1 using a small molecule inhibitor of E1 activation, TAK-243 (MLN7243). We postulated that if E1 was involved in CBP and MLL1 degradation, then inhibition of E1 with TAK243 would rescue the loss of these molecules upon treatment with E7386. These studies are currently in progress. Our studies are aimed at understanding the biology of E7386 for its future application to improve treatments for OSCC patients.

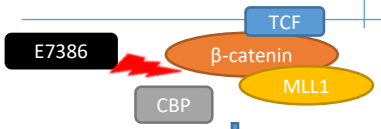




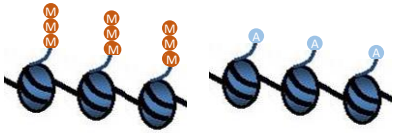
β-catenin/CBP acts as transcriptional co-activator to drive aggressive cancer phenotypes in OSCC cells



E7386 specifically inhibits β-catenin/CBP interaction and promotes cell differentiation



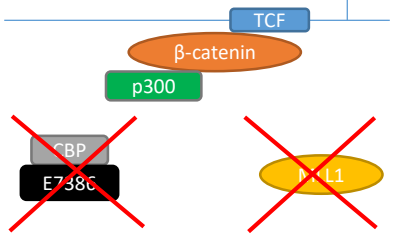
β-catenin/CBP drives H3K27 acetylation while β-catenin/CBP/MLL1 is responsible for H3K4 trimethylation, which together promote an open chromatin structure and transcriptional activation of cancer stem cell (CSC) genes, including epithelial mesenchymal transition (EMT), unstable or transient cell phenotypes.

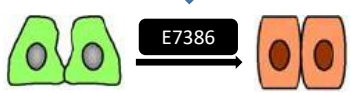


H3K4me3

H3K27ac

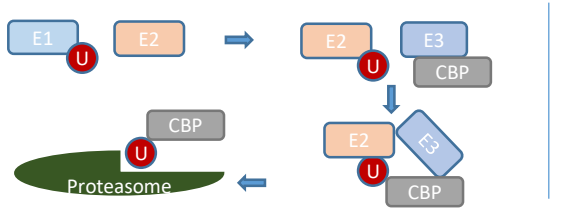
β-catenin state-level remains constant but the abundance of CBP and MLL1 is drastically decreased



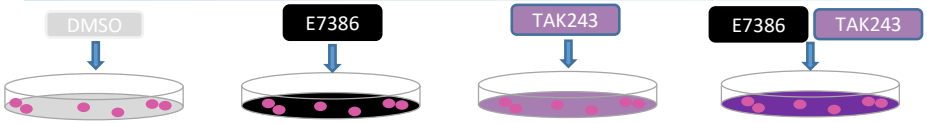


What is the mechanism for the loss of CBP and MLL1 in response to the inhibition of β-catenin-CBP?

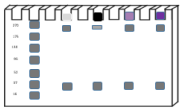
We hypothesize that the ubiquitin-proteasome pathway is responsible for the degradation of CBP and MLL1 when not in complex with β-catenin.



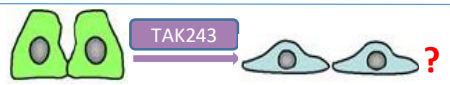
TAK-243 (MLN7243) is a small molecule inhibitor of E1 activation



- HSC3 cells seeded in DMEM media (10% FBS, 1% rhodamine, 1% pen-strep) until 50%^ density;
- Cell plates receive 4 types of treatment: DMSO (0.1%), E7386 (1 μM) only, TAK243 (10-80 nM) only, and mixture of E7386 and TAK243.

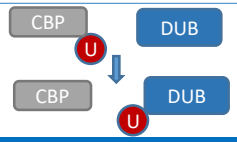


- Protein mixtures are extracted after 48 hours treatment and tested by western blot;
- Specific target proteins are separated based on differences in molecular weight (CBP ≈ 265 kDa; MLL1 ≈ 212 kDa);
- GAPDH (≈ 37 kDa) is a housekeeping enzyme often been used to normalize.



- OSCC cells are expected to show more aggressive phenotypes, such as EMT

- OSCC cells are expected to be resistant to the inhibitory effects of E7386 due to the maintenance of CBP and MLL1 abundance



- DUB (deubiquitinating enzyme) reverses ubiquitination process(es), detaches ubiquitin from protein and prevents protein degradation;
- DUB is going to be invested in future study.

Citations

Alamoud, K. A. and M. A. Kukuruzinska (2018). "Emerging Insights into Wnt/β-catenin Signaling in Head and Neck Cancer." J Dent Res 97(6): 665-673.
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