p300/CBP inhibitor CCS1477 targets 22Rv1 prostate tumour AR and c-Myc gene expression in vivo.

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Introduction

- Histone acetyl transferases (HAT) p300 and CREB binding protein (CBP) are co-activators of transcription factors that contribute to prostate tumour progression including androgen receptor (AR), HIF1α, and c-Myc.

- A large proportion of AR regulated gene expression has been dependent on CBP/c-Myc interactions and disruption of the interaction decreases AR regulated gene expression.

CCS1477 is a potent, selective inhibitor of the bromodomain in p300/CBP that has been shown to inhibit prostate tumour cell proliferation in vitro and tumour growth in vivo.

1. CCS1477 significantly inhibited 22Rv1 tumour growth with prolonged tumour growth inhibition after cessation of treatment.

2. CCS1477 significantly modified the expression of a subset of expressed genes.

- VEGF was significantly downregulated in CCS1477 treated tumours.

3. CCS1477 p300/CBP inhibition both increased and reduced the expression of similar numbers of genes.

- With and down-regulated genes in CCS1477 treated tumours with an adjusted p-value <0.05 for each comparison with the genes with >2 fold change and adjusted p-value <0.05 is shown in the table below.

4. CCS1477 potentially disrupts circadian regulation.

- c-Myc and AR are key regulators of the circadian clock that regulates the expression of ~12% of all expressed genes. CCS1477 expression of CLOCK/RBM15 was the most downregulated gene at day 7.

5. CCS1477 inhibited angioderated gene expression.

- Treatment with CCS1477 inhibited gene expression including KDM3A, VEGF.

6. CCS1477 inhibited c-Myc, VEGF and HIF regulated gene expression.

- c-Myc expression was downregulated at day 7 and day 28 and was increased with tumour regrowth at day 52. Myc and AR was downregulated at day 28. VEGF was downregulated at all timepoints.

7. CCS1477 significantly upregulated histone expression.

- Histone RNAs comprised the core nucleosomes (H2A, H1, H4) and linker histone (H5) were among the most highly upregulated targets (examples shown here). Accumulation of histories may be a contributor to growth suppression through genomic instability and cell cycle dysregulation.

8. CCS1477 upregulated non-coding RNAs.

- Non-protein coding RNAs representing core components of the spliceosome were significantly increased at all timepoints expressing a widespread silencing of gene translation. c-Myc has been implicated as a master regulator of ribosome biogenesis and spliceosome function.

Potential impact of CCS1477 on key pathways in prostate cancer

Methods

- Gene expression analysis was performed using AFFymetrix Claria 3 microarrays. Data QC and statistical analysis was performed to identify potential outliers using AffyBatch's 3Statistics, Procrustes correlation, Sudderlin distance and Age-correction Smirnov tests. Pairwise comparisons were performed to identify differentially expressed genes.

- CCS1477 was administered daily by gavage to 22Rv1 tumour bearing animals. Tumours were harvested after 12 hours following treatment.

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- Fold change cut-off values of 1.5 and 2 and a false Discovery Rate (FDR) p-value <0.05 were used to identify significant changes in gene expression.

Conclusions

- Inhibition of p300/CBP activity in 22Rv1 prostate tumour xenografts resulted in widespread aberration of gene expression.

- Histone and non-protein coding RNAs were significantly upregulated at all timepoints suggesting disruption of transcription and translation in addition to the impact of p300/CBP inhibition on protein and histone synthesis.

- Downregulation of VEGF expression together with downregulation of histone related genes KDM3A, PLOD2 and P4HA1 suggest that angiogenesis is inhibited in response to CCS1477 treatment.

- Response to p300/CBP inhibition and the prolonged duration of tumour growth inhibition following treatment withdrawal likely results from a complex network of key pathways regulated by AR, c-Myc, and chromatin disruption.

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