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# Evaluating Transcription Factor Networks as Targets for the Treatment of Neuroendocrine Tumors

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**BACKGROUND:** ASCL1 is a transcription factor that is highly expressed in neuroendocrine tumors (NETs) including, gastrointestinal carcinoids, pheochromocytoma, medullary thyroid carcinoma, NE prostate cancer and small cell lung cancer (SCLC). ASCL1 expression is particularly critical for the proliferation of some gastrointestinal carcinoids and SCLCs. Inhibiting ASCL1 transcriptional activity may thus be a valid therapeutic strategy for these NETs. However transcription factors (TFs) have been historically difficult to target. Given that TFs belong to complex regulatory networks, we propose to identify and target multiple components within an ASCL1-transcriptional network rather than ASCL1 alone. We have previously detected ASCL1-bound genomic regions associated with the active chromatin epigenetic mark, H3K27Ac in SCLC cells. Here we report the identification of an ASCL1-containing transcriptional network in SCLC cells and evaluate its targeting by the transcriptional inhibitor, mithramycin.

**METHODS:** Chromatin immunoprecipitation followed by high-throughpout sequencing (ChIP-seq) were conducted by immunoprecipitating chromatin from NCI-H2107 and NCI-H69 cells (5X107 cells) with 10 µg H3K27Ac antibodies (Abcam-Ab4729). ChIP-seq libraries were sequenced on an Illumina High-Seq2000. siRNA transfections, immunoblotting and MTS-cell proliferation assays were conducted using standard procedures.

**RESULTS:** 82 overlapping TF genes, including lineage-specific TF genes for, ASCL1, FOXA2 and NFIB, were found associated with the active chromatin epigenetic marks H3K27Ac in SCLC cell lines. This suggests ASCL1, FOXA2 and

NFIB may belong to the same transcriptional network. siRNA-mediated knock-down of ASCL1 leads to decreased FOXA2 levels but it has no effect on NFIB levels, implicating ASCL1 as a transcriptional regulator of FOXA2 but not NFIB. Mithramycin stops cell proliferation and reduces ASCL1, FOXA2 but not NFIB protein levels.

**CONCLUSION:** Our studies identify the beginning of a transcriptional network necessary for SCLC proliferation in which ASCL1 regulates FOXA2. Targeting multiple TFs within a network with mithramycin can stop cell proliferation and as such suggests a therapeutic strategy for NETs.