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The Lineage Transcription Factors ASCL1, NKX2-1, and PROX1 Form a Regulatory Network and Control the Expression of the Ion Channel, SCN3A, in Small Cell Lung Cancer.

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BACKGROUND: Heterogeneity between tumors is a barrier to the treatment of small cell lung cancer (SCLC). Four molecular subtypes, SCLC-A, -N, -Y and -P have been defined but their biology is not well understood. Identification of subtype-specific vulnerabilities could facilitate the development of targeted therapies. The lineage transcription factor (TF) ASCL1 is expressed in over 70% SCLC and defines SCLC-A. We have found ASCL1 and two other TFs, NKX2.1 and PROX1, are enriched at super-enhancers in patient-derived SCLC-A cell lines and xenografts. Here we investigate the functional interactions between ASCL1, NKX2.1 and PROX1.

METHODS: ASCL1, PROX1, and NKX2.1 were immunoprecipitated from NCI-H2107 cells and immune pellets analyzed by immunoblotting. For chromatin-immunoprecipitations, ASCL1, PROX1, and NKX2.1 were isolated from NCI-H2107, NCI-H889 and NCI-H128 cells and analyzed by RNA-seq. For functional analyses, cells were transfected with siRNAs and analyzed 48-72h after transfection. Cell survival was measured using WST assays, gene-expression was analyzed by next-generation sequencing and protein expression by immunoblotting. To assess SCN3A subtype-specificity, a 1:1 ratio of NCI-H2107 cells (SCLC-A) and NCI-H524 cells (SCLC-N) were mixed, stained with SCN3A antibodies and FACS-sorted.

RESULTS: 1) ASCL1 co-immunoprecipitates with PROX1 and NKX2.1. 2) ASCL1, PROX1 and NKX2.1 share ~2000 binding sites on the SCLC-A genome. 3) The 3 TFs regulate ~1010 common genes, including the ion channel SCN3A. 4) The 3 TFs cross-regulate each other. 5) ASCL1, PROX1 and NKX2.1 knock-down results in loss of SCN3A and reduction in SCLC-A cell survival. 6) SCN3A knock-down does not reduce cell survival. 7) SCLC-A cells can be isolated from a cell mixture using SCN3A antibodies.

CONCLUSION: ASCL1, PROX1 and NKX2.1 form a TF network and regulate SCN3A expression. SCN3A is a specific marker for the SCLC-A subtype. Our strategy can be used to identify subtype-specific markers for the detection of the SCLC-A subtype.

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