Analysis of neuroblastoma super-enhancer landscape identifies two distinct malignant cell types

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Neuroblastoma is a tumor of the peripheral sympathetic nervous system [1], derived from multipotent neural crest cells (NCC). It is a pediatric cancer, with the average age at diagnosis of 18 months. Neuroblastoma genomes are characterized by relatively few recurrent driver mutations. The most frequently mutated neuroblastoma-related gene, *ALK*, is found to be altered in less than 15% of cases at diagnosis. This scarcity of identified driver mutations in neuroblastoma leaves a possibility of the “driving” role of non-genetic, i.e. epigenetic changes in oncogenic processes of this pediatric cancer.

Indeed, latest studies have demonstrated that tumorigenesis of many cancers is associated with considerable epigenetic modifications: changes in the chromatin states and DNA methylation. Changes in chromatin states in cancer include, in particular, formation of *de novo* enhancers and super-enhancers (i.e. long active chromatin regions encompassing tens of Kb and comprising a dozen of enhancer elements) and enhancer hijacking [2,3].

Here we analyzed the enhancer and super-enhancer landscape of neuroblastoma and detected transcription factors that constitute the core neuroblastoma regulatory circuitries and drive expression of genes determining cell identity in neuroblastoma and affecting cell phenotype. For this study, we used 20 neuroblastoma cell lines and two patient-derived mouse xenografts. We demonstrated that integrative analysis of ChIP-seq profiles for the acetylation of lysine 27 of the histone H3 and gene expression data can point to the key transcriptional regulators of neuroblastoma. We also defined two neuroblastoma identity subtypes. The most common one was associated with amplification or high expression of the MYCN oncogene and high activity of several noradrenergic transcription factors. The other subtype was correlated with the activity of the AP-1 complex. Moreover, we identified potential enhancer and super-enhancer regions active in each subtype, and reported that the over-expressed transcription factor PHOX2B driven by its super-enhancer is one of the important transcriptional drivers of the first neuroblastoma subtype. We showed that depletion of PHOX2B leads to an inhibition of neuroblastoma cell growth.

To analyze ChIP-seq data we used a method we specifically develop to process ChIP-seq profiles generated from cancer cells [4]. This method, HMCan, takes into account copy number alterations ubiquitously present in cancer cells; it also corrects for the library size and GC-content bias.

To predict super-enhancer regions we used an extended version of the ROSE tool – LILY

(http://boevalab.com/LILY/). Both ROSE and LILY detect super-enhancer regions using the H3K27ac signal. In contrast to ROSE, LILY takes into account copy number alterations, which distort the H3K27ac signal in cancer cells, and provide more accurate annotations of super-enhancers in regions of copy number loss and gain.

To detect possible drivers of the super-enhancer landscape in neuroblastoma, motif discovery with the i-cisTarget method [5-6] was applied to regions corresponding to valleys of H3K27ac peaks located in super-enhancers. Valleys in H3K27ac profile are likely correspond to binding sites of transcription factors, i.e. regions devoid of nucleosomes. To detect these valleys we used an *ad hoc* script based on smoothed H3K27ac profiles normalized by HMCan for the GC-content bias.

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