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EFFECTS OF IN VITRO CULTURE ON EPIGENETIC CONTROL OF BIVALENT DOMAINS (H3K4ME3 E H3K27ME3) AND EXPRESSION OF NEURONAL GENES IN CORTICAL NEURONS

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In vitro culture has the potential to alter chromatin status and gene expression in several cell types. However, cell culture is a powerful tool to study live systems, allowing faster and more specific assessments than other approaches. H3k4me3 and H3k27me3 are histone modifications associated to permissive and repressive chromatin states, respectively, and together they represent bivalent domains. The purpose of this study was to address how in vitro culture of cortical neurons affects H3k4me3 and H3k27me3 markers and mRNA expression in neuronal genes. To assess that, neural cortex samples were collected from E.18 mouse C57bl6 fetus and dissociated into single cell suspension. Cells were immediately fixed/frozen (GI) or cultured for 48h in Neurobasal medium and then fixed/ frozen (GII). For Experiment 1 (n=3 per group), chromatin immunoprecipitation (ChIP) was performed using a kit, accordingly to manufacturer instructions (EzChIP, Millipore), followed by qPCR for neuronal genes using standard curve method. Results are presented as total amount of DNA immunoprecipitated with H3k4me3 and H3k27me3 antibodies for each gene, subtracted from IgG negative control and divided by non IgG precipitated input. For experiment 2 (n=9 per group), gene expression assessment by qPCR was performed using standard curve method, and results are presented as total mRNA estimated for each gene. GAPDH was used as endogenous control gene. Results from GI and GII groups were compared using T-test. In experiment 1, we observed that although the amount of DNA immunoprecipitated (IP) for H3k4me3 and H3k27me3 was variable between groups, no significant difference was detected for genes Ascl1 (H3k4me3: GI 0,81±0,49; GII 31,85±42,95/ H3k27me3: GI 0,75±0,63; GII 29,78±45,02), Nestin (H3k4me3: GI 5,60±8,92; GII 0,95±1,05/ H3k27me3: GI 0,76±0,04; GII 5,44±2,76) and NeuroD (H3k4me3: GI 0,30±0,09; GII 0,20±0,34/ H3k27me3: GI 1,1±1,58; GII 0,01±0,003). However, for Neurog1 we observed 1,91 fold enrichment ($p<0.05$) on H3k27me3 levels in GII (H3k4me3: GI 0,11±0,09; GII 0,17±0,19/ H3k27me3: GI 2,66±1,61; GII 5,09±0,29*). Regarding the pattern between H3k4me3 and H3k27me3 IP levels in each group, we observed similar distribution for Ascl1 (similar levels for H3k4me3 and H3k27me3) and NeuroGI (higher H3k27me3 levels); for the other genes, an inversion was detected: Nestin (higher H3k4me3 for GI and higher H3k27me3 for GII), and Neurod (higher H3k27me3 in GI and higher H3k4me in GII). In experiment 2, expression profile remained unaltered between groups for genes: Neurog1 (GI: 8,08±0,86; GII: 8,69±1,42) and NeuroD (GI: 21,5±1,07; GII: 18,97±1,32). However, Ascl1 expression was higher ($p<0.05$) for GII (GI: 2,84±0,13; GII: 3,68±0,27*) and Nestin expression was decreased ($p<0.05$) in GII (GI: 6,68±0,59; GII: 4,87±0,59*). We concluded that *in vitro* culture can affect H3k4me3 and H3k27me3 patterns and gene expression of cortical neurons.

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