Poster code: GHM0004

HMGN3a localization and HMGNs expression in P19 embryonal carcinoma cells

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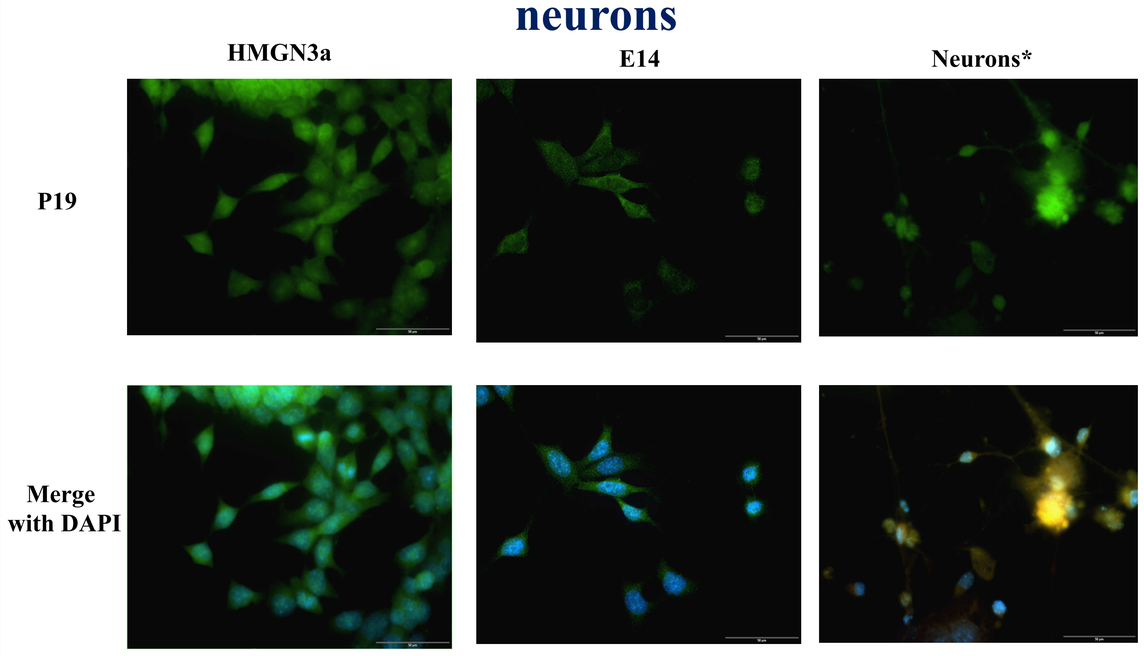
Introduction

HMGN (High Mobility Nucleosome Binding) are nucleosome-binding proteins that play important roles in epigenetic processes and gene expression. Previous studies have shown HMGN3a protein to be nuclear, but a recent study has shown HMGN3 to be mostly present in the cytoplasm of P19 cells, which are malignant cells derived form teratocarcinomas that originate in gonads, but still resemble embryonic stem cells (ESCs). Alongside, studies revealed that the expression level of HMGN proteins is tightly related to the differentiation process of both *Xenopus* and mice and that expression of HMGN1 and HMGN2 decrease during erythropoiesis, chondrogenesis, and myogenesis. Herein we address the cellular localization of HMGN3a in mouse ESCs, undifferentiated P19 cells, and neurons derived from differentiated P19 cells. Additionally we found that the expression of HMGN1 and HMGN2 proteins decreased at the late stages of neuronal differentiation *in vitro*, applying an adherent culture system in embryonic carcinoma cells (ECCs, P19; Nakayama et al, 2014*). Thus, this adherent culture system provides a model to investigate the role of HMGN proteins and epigenetic processes during neuronal differentiation.

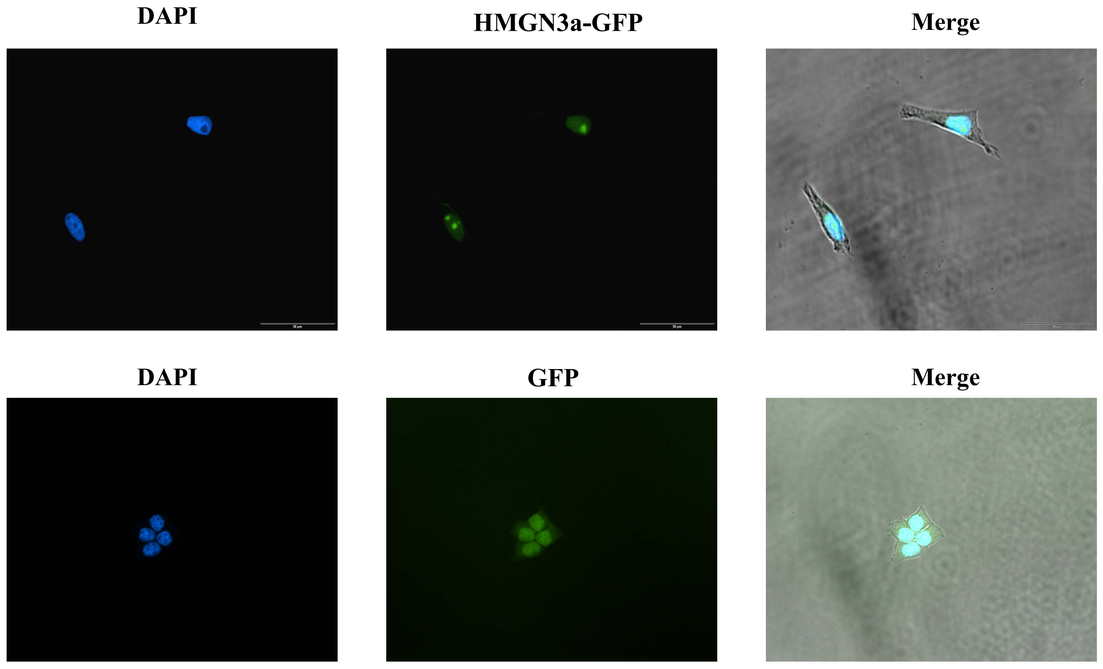
Immunofluorescence signal of HMGN3a in P19, E14 and neurons



*Merge with DAPI (blue) and MAP2 (red)

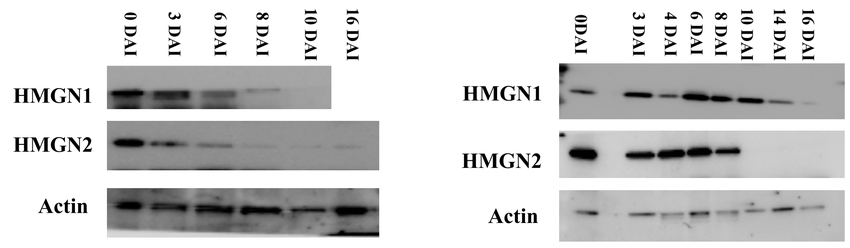
• Immunofluoresence data showed HMGN3a is present in the cytoplasm and nucleus of P19 cells and P19-derived neuronal cells. HMGN1 and HMGN2 were predominantly localized in the nucleus of all the cells studied, as expected from previous work. The same immunofluorescence results were observed for E14 cells.

GFP-tagged HMGN3a in P19 cells

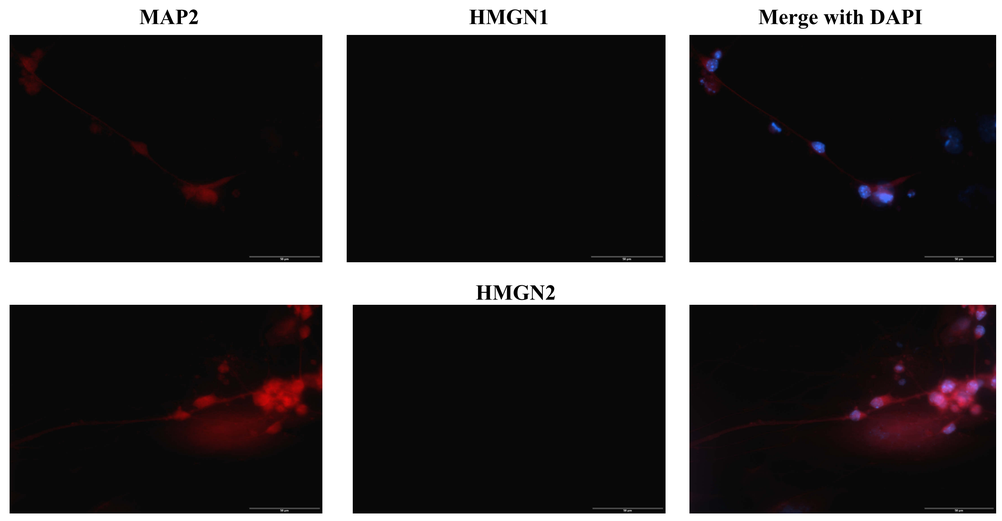


Transfection of HMGN3a-GFP and GFP showed the protein clearly localised to the nucleus. This data suggests that HMGN3 is actually localised in the nucleus, and that artefacts due to antibody specificity or the immunofluorescence technique gave rise to the cytoplasmic signal.

HMGN1 and HMGN2 expression is reduced during neuronal differentiation *in vitro*



Western blot data from two different experiments indicate a reduction in the expression of HMGN1 and HMGN2 after neural induction. Both proteins are found at high levels in undifferentiated ECCs, and are nearly undetectable in terminal stages of differentiation.



Immunofluorescence indicate that HMGN1 and HMGN2 protein levels decrease as cell differentiate into post-mitotic neurons. This data is consistent with mRNA expression patterns from the Allen Brain Atlas, which shows high levels of HMGN expression in regions of proliferating neural stem and progenitor cells, but reduced HMGN expression in post-mitotic neuronal cells.

Conclusions and future work

•These results are consistent with a role for HMGN proteins in neural stem cells, and this is the subject of ongoing investigations.

•New immunofluorescence assays with monoclonal antibodies would extinguish the artefacts that were observed with polyclonal antibodies, and corroborate the results obtained with the fusion protein HMGN3a-GFP.

Reference

• Nakayama Y, Wada A, Inoue R, Terasawa K, Kimura I, Nakamura N, Kurosaka A (2014). A rapid and efficient method for neuronal induction of the P19 embryonic carcinoma cell line. *Journal of Neuroscience Methods*. 227; 100–106.

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