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Structural insights on melanocortin 5 receptor targeting to cell surface

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The melanocortin 5 receptor (MC5R) is a G-protein coupled receptor (GPCR) with a typical seven-transmembrane-domain structure. It is assumed that all GPCRs undergo several post-transcriptional modifications during synthesis and folding until finally target to the cell membrane [1]. The available information about the specific domains responsible for the correct targeting of the melanocortin receptors to the cell surface is scarce. Regarding MC5R, it was shown that the first 20 aminoacids of the human receptor can be deleted without affecting the ligand binding affinity, but further deletions of the N terminus resulted in total loss of binding [2]. These results were not directly correlated with the cell surface expression levels of the receptor. Thus, we aim to define the specific MC5R domains important to its correct trafficking and signaling, and preliminary results are here presented.
The expression of the rat MC5R fused to the green fluorescent protein (GFP) was achieved in the HEK 293 cell line. We recently showed a correct targeting to cell surface and the attaining of a functional receptor when the GFP tag was located at the MC5R C-terminal (MC5R-GFP) [3]. In contrast, the MC5R construct containing the GFP at the N-terminus (GFP-MC5R) displays lower expression levels and was unable to target to the membrane efficiently (Fig.1). Since the attachment of a 27 kDa GFP polypeptide to the receptor N-terminus could impair its correct folding and the consequent membrane insertion, we therefore generated constructs of c-myc (8 kDa) tagged at the N- and C-terminal MC5R. Once more, MC5R-c-myc was expressed on the cell surface but c-myc-MC5R was strongly retained inside the cell (Fig. 2A). To further investigate whether the inability to traffic to the plasma membrane was cell-related, we use HeLa cells, transiently expressing the N- and C-terminal-c-myc-tagged MC5R constructs, and a similar expression pattern was observed (Fig. 2B). Our results highlight a crucial role for the N-terminus domain on MC5R membrane targeting and expression, in opposition to the findings that short N-terminal epitope tagging of MC1R, MC2R, or MC4R does not affect receptor trafficking or function [4]. Further experiments will be necessary to clarify the precise aminoacids from the N-terminus of the receptor MC5R that are fundamental to the receptor cell-surface expression.

References

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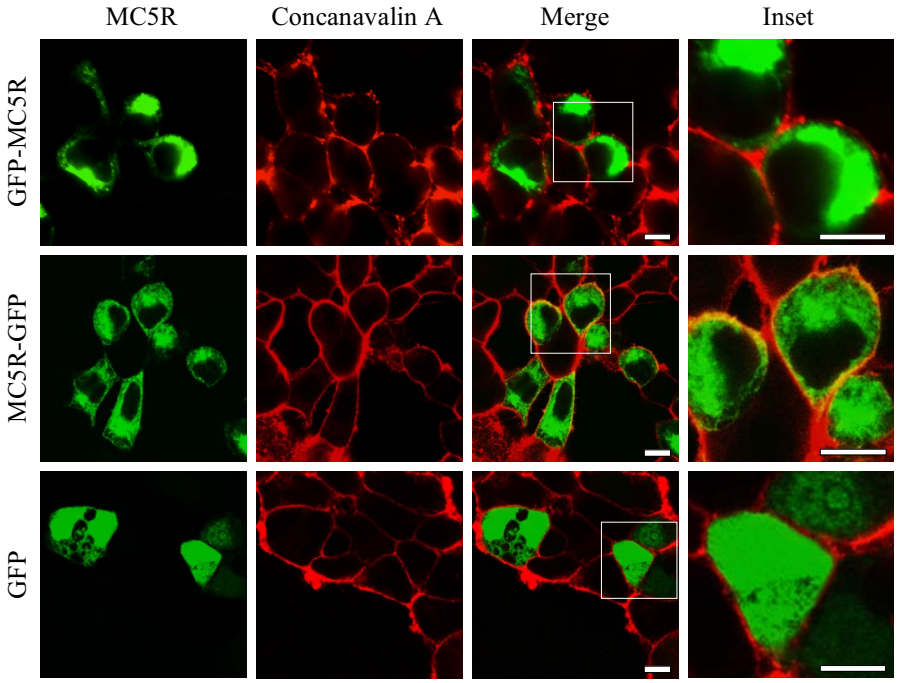

Fig 1. Confocal immunofluorescence imaging of HEK293 cells transiently transfected with C- and N-terminus GFP-tagged MC5R or GFP alone. Cell surface was labeled with Alexa®594-conjugated concanavalin A prior to cell fixation (bar: 10µm).

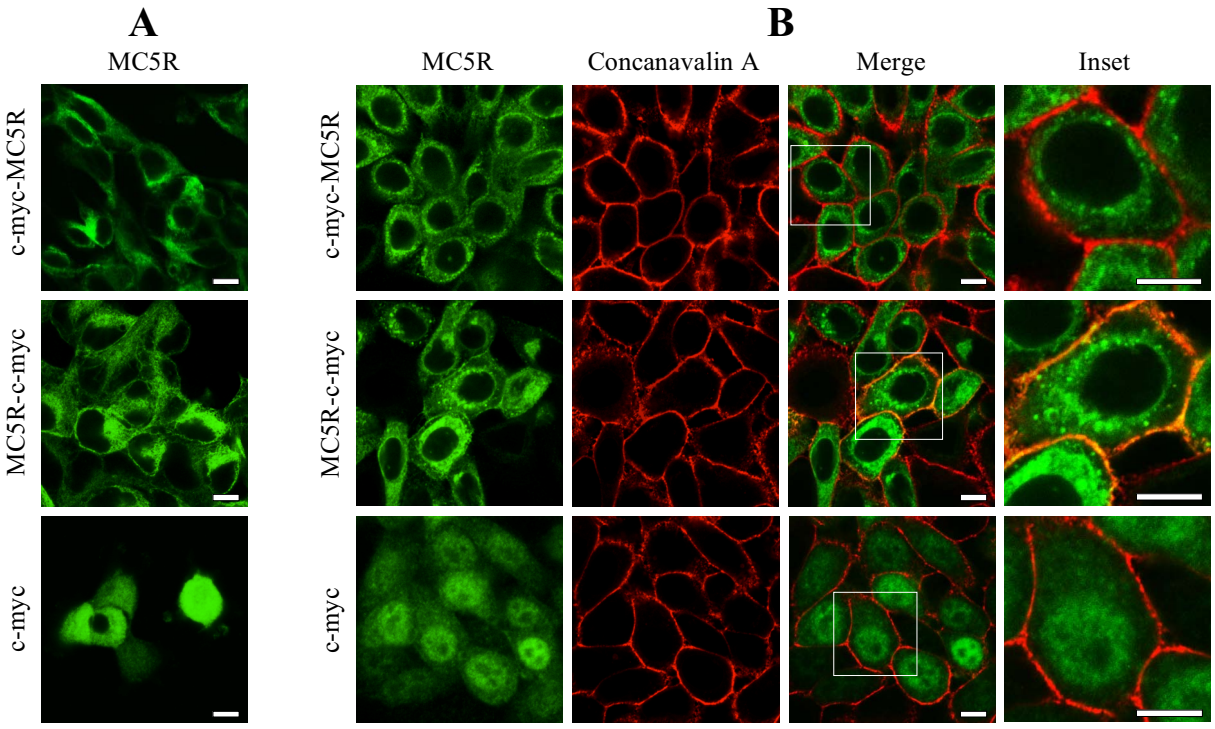

Fig. 2. Transient expression of c-myc-tagged MC5R in HEK293 (A) and HeLa (B) cell lines. MC5R localization was assessed by using an anti-c-myc antibody and a Alexa®488-conjugated secondary antibody. Plasma membrane was labeled with Alexa®594-conjugated concanavalin A and the images were acquired using a confocal microscopy (bar: 10µm).