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polarization, which are regulated by the small GTPase Rac1. In the absence of syndecan-4 (SDC4) proteoglycan, the activity of Rac1 is increased. SDC KO mice are unable to regenerate damaged muscle; however, the clear mechanism has not been investigated. So our aim was to study the role of SDC4 and Rac1 in myoblast migration.

Methods: We used shRNA to silence SDC4 in C2C12 mouse myoblasts. After live-cell microscopy, we analyzed cell motion (n=84-150 cells/cell line) with FIJI image analysis program and CellTracker. Rac1 activation was inhibited by NSC23766 treatment (50 μ M).

Results: In the silenced cell lines, the overall route (non-transfected vs. scrambled vs. shSDC4#1 vs. shSDC4#2: 527,5 $\pm$ 10,72 vs. 512,4 $\pm$ 14,59 vs. 262,9 $\pm$ 6,63 vs. 281,7 $\pm$ 8,56 μ m), average speed (0,49 $\pm$ 0,010 vs. 0,48 $\pm$ 0,014 vs. 0,25 $\pm$ 0,01 vs. 0,27 $\pm$ 0,008 μ m/minute) and maximal speed of the cells (2,02 $\pm$ 0,05 vs. 2,06 $\pm$ 0,06 vs. 1,2 $\pm$ 0,04 vs. 1,34 $\pm$ 0,06 μ m/minute) have significantly decreased compared to both non-transfected and scrambled expressing cells. Rac1 inhibitor NSC23766 treatment has significantly decreased all these previously examined parameters.

Conclusions: Both SDC4 silencing and inhibition of Rac1 activity decrease the migration of myoblasts. Thus neither too high nor too low Rac1 level allows effective migration. Our results may contribute to the understanding of the essential role of SDC4 in skeletal muscle development and regeneration.

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I/5 THE MEASUREMENT OF THE DIPOLE POTENTIAL WITH A NOVEL HIGH-THROUGHPUT FLOW CYTOMETRIC METHOD

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Keywords: dipole potential, hypercholesterolemia, di-8-ANEPPS, 3-hydroxyfl

Aims: The least-known component of membrane potentials, the dipole potential (DP) originates from the preferential alignment of interfacial water dipoles and dipolar segments of phospholipids and sterols, resulting in a large positive ($\sim$ 300mV) potential creating an immensely strong intramembrane electric field. Consistently, the DP was shown to influence a multitude of membrane-coupled events (e.g. gating of voltage-gated ion channels, ligand-induced clustering and activation of ErbB, etc). The magnitude of the DP is mainly determined by the lipid composition of the cell membrane.

Methods: Since the DP is entirely located within the plasma membrane, it is difficult to measure directly. Di-8-ANEPPS, an electrochromic, voltage-sensitive fluorophore, is most commonly used to estimate its magnitude via ratiometric excitation methods using spectrofluorometry or fluorescence microscopy. We have newly developed and tested an emission ratiometric method using flow cytometry, based on a novel group of fluorophores, 3-hydroxyflavons.

Results: Using human lymphoblastoid and monocytoid cell lines, and human peripheral blood mononuclear cells, we demonstrate the applicability of our method using conventional DP-modifying 6-ketocholestanol and phloretin treatments. Furthermore, loading of cells with cholesterol or 7-dehydrocholesterol results in dose-dependent increases in the magnitude of DP. In our ongoing studies, we are examining the effects of polyunsaturated fatty acids, and determining the alterations in the DP in hypercholesterolemic patients compared to control individuals.

Conclusions: In conclusion, we have developed a novel method to determine changes in the magnitude of DP in various pathological conditions, which is suitable for high-throughput measurements using a flow cytometer.

I/6 CHANGES IN THE COLOCALIZATION BETWEEN POTASSIUM CHANNELS AND LIPID RAFTS IN RESPONSE TO CHOLESTEROL, 7-DEHYDROCHOLESTEROL AND CE

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Keywords: Kv1.3, Kv10.1, cholesterol, ceramide, lipid rafts

Aims: The preferential lipid raft localization has been demonstrated in voltage-gated potassium channels Kv1.3 and Kv10.1. Lipid raft localization significantly modifies structural and functional properties of these ion channels. Our previous studies demonstrated that treatment with the major lipid raft constituents cholesterol or ceramide exerts remarkable effects on electrophysiological parameters of Kv1.3 and Kv10.1. However, the exact molecular mechanisms of these alterations are unknown.

Methods: In our experiments, we analyzed the effects of cholesterol, 7-dehydrocholesterol (7DHC) and ceramide on the lipid raft localization of Kv1.3 and Kv10.1. For microscopic analysis of the colocalization of the channels and lipid rafts, tsA_201 cells were transiently transfected with Kv1.3 or Kv10.1 proteins. After loading of the cells with cholesterol, 7DHC or ceramide, channels and rafts were visualized by confocal microscopy. During image analysis, the Pearson correlation coefficients between channel and raft marker signals were determined.

Results: First, we tested our method by analyzing the colocalization between two known raft markers, cholera toxin-B subunit and GFP-GPI. As expected, highly positive values of the correlation coefficient were found. Correlation coefficients of similar magnitude were determined between intensities of the raft marker and the antibodies against ion channels, demonstrating the preferential raft localization of Kv1.3 and Kv10.1. Furthermore, the extent of co-localization significantly increased in response to treatments with cholesterol, 7DHC and ceramide in both channels.

Conclusions: In conclusion, we have demonstrated that changes in the distribution of Kv1.3 and Kv10.1 between different membrane microdomains might at least partially mediate the effects of cholesterol and ceramide on these channels.

IS. Burning topic: Working in the UK as a doctor, collaboration between HMAUK and HMAA

IS/1 COLLABORATION BETWEEN HMAA AND ITS SISTER ORGANISATION, HMA-UK, AND MEDICAL MIGRATION IN EUROPE

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