

Pulmonary epithelial cancer cells, and their exosomes, metabolize myeloid cell derived leukotriene C₄ to leukotriene D₄

Supplement

JLR/2016/066910, revised

Ana Lukic¹, Jie Ji², Helena Idborg³, Bengt Samuelsson¹, Lena Palmberg², Susanne Gabrielsson⁴, and Olof Rådmark¹

¹Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II

² Lung and Airway Research, Institute of Environmental Medicine

³Department of Medicine Solna, Rheumatology Unit

⁴Department of Medicine Solna, Unit of Translational Immunology

All at Karolinska Institutet, 171 77, Stockholm, Sweden

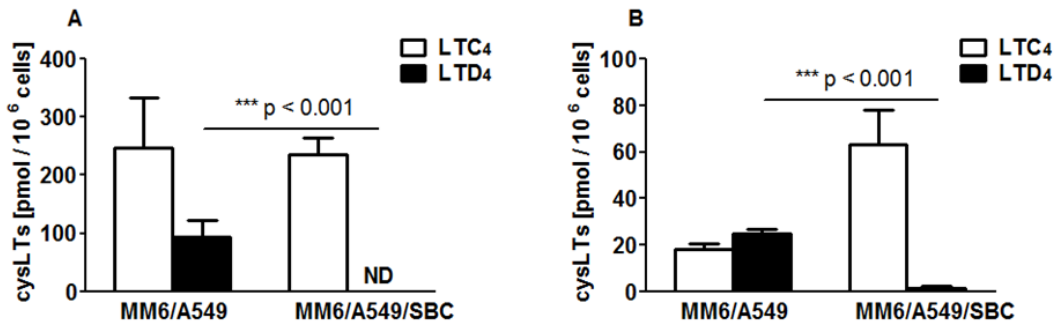


Fig. S1

Supplemental Figure S1. The effect of serine borate complex (SBC, 10mM) on conversion of LTC₄ to LTD₄ (GGT activity) was determined in MM6/A549 coculturations. A) Cocultures were pretreated with SBC for 10 minutes before incubation with LTA₄ (20μM, 5 min). Following extraction, CysLTs were analyzed by HPLC (n=3). B) Cocultures were pretreated with SBC for 5 minutes before additions of PMA (100nM, 10 min) followed by ionophore A23187 (5μM, 10 min). (n=7). Following extraction, CysLTs were analyzed by HPLC. The results are given as Mean ± SE, student's t-test. ND means not detected.

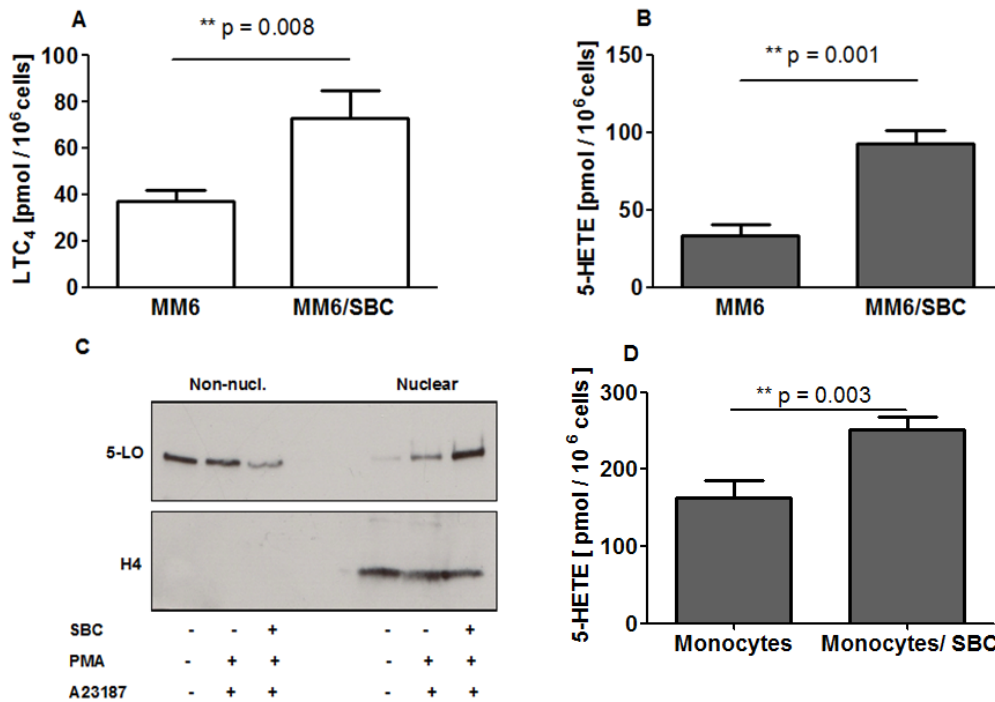


Fig. S2

Supplemental Figure S2. The effect of serine borate complex (SBC, 10mM) on 5-LO activity and translocation in MM6 cells. A-B) Differentiated MM6 cells were pretreated with SBC for 5 minutes before additions of PMA (100nM, 10 min) followed by ionophore A23187 (5 μ M, 10 min). Following extractions, LTC₄ (A) and 5HETE (B) were analyzed by HPLC (n=6), student's t-test. C) Translocation of 5-LO to the nuclear fraction. 4x10⁶ cells were incubated as described above, and cytosolic and nuclear fractions were prepared, see Methods. Pair-wise samples of non-nuclear and nuclear fractions, corresponding to identical cell numbers, were loaded on SDS-PAGE and immunoblotting was performed for 5-LO and histone H4 (control for loading of nuclear fractions). Similar results were observed in three different experiments. D) Monocytes prepared from peripheral blood cells were pretreated with SBC for 5 minutes before additions of PMA (100nM, 10 min) followed by ionophore A23187 (5 μ M, 10 min). Following extractions 5-HETE was analyzed by HPLC. Cells from two donors were analyzed in duplicates. Student's t-test.

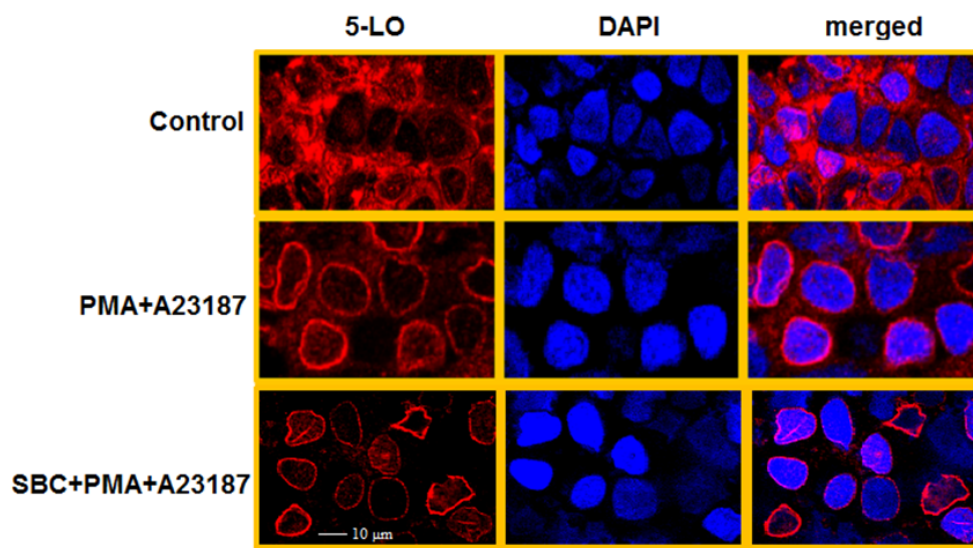


Fig. S3

Supplemental Figure S3. Immunocytochemistry of 5-LO in MM6 cells. Cells were incubated as described in legend to supplemental Figure S2. Immunofluorescence images (see methods) show single staining for 5LO (red, Cy3), nucleus (blue, DAPI) and merged pictures. Bar = 10μm. Similar data were obtained in two additional independent analyses.