COMMUNICATION BETWEEN MACROPHAGES AND CANCER CELLS ORCHESTRATES LIPID MEDIATOR BIOSYNTHESIS

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Introduction: The tumor microenvironment plays a crucial role in the progression of cancer. It is composed by stromal cells, endothelial cells and immune cells. The latter are mostly macrophages (so called tumor-associated macrophages TAM) that depending on their polarization status can suppress or promote the tumor growth (M1and M2, respectively). TAM have predominantly an M2-like phenotype and are responsible for the unresolved inflammation that surrounds the tumor. In our body, macrophages are a major source for lipid mediators (LMs) with a variety of biological functions, advantageous or deleterious for tumors. LMs are bioactive small molecules mostly produced by cyclooxygenases (COXs) or lipoxygenases (LOXs) that orchestrate the inflammatory reaction from the onset to the resolution. Given the co-existence of cancer cells and TAM in the tumor milieu, we investigated how the communication of human monocyte-derived macrophages (MDM) with cancer cells affects LM biosynthesis using LM metabololipidomics.

Materials and methods: Cocultures of cancer cells (lung carcinoma A549, colon adenocarcinoma HT-29, leukemia HL-60 cells) or HUVECs with macrophages was performed in a Boyden chamber system combining 6well plates and Falcon Cell Culture inserts with 0.4μM pore size. Cancer cells or HUVECs were seeded in the well plate. After 2h, inserts were installed on top of the wells, immediately followed by the addition of macrophages. 2h later, IL-4(20 ng/ml) was added to the Boyden chamber system. Cells were co-cultured (or single-cultured in case of the controls) for another 48h. Then, cell populations were separated from each other and stimulated with E. coli (serotype O6:K2:H1) at a ratio of 1:50 (human cells:E. coli) for 90 min at 37°C or with 0.5μM Ca^{2+} -ionophore A23187for 10 min at 37°C. Supernatants (2ml) were transferred to 3ml of ice-cold methanol containing deuterium-labeled internal standards (200 nM d_8-5S-HETE, d_4-LTB_4, d_5-LXA_4, d_5-RvD2, d_4-$PGE_2$and 10 μM d_8-AA) to facilitate quantification. LM profile analysis was done by UPLC-MS/MS. The expression of LM biosynthetic enzymes was evaluated by Western blot.

Results: Co-culture of MDM with different cancer cells (i.e. A549, HT-29) but not endothelial cells (HUVEC) substantially increased formation of 5-LOX-derived LM (i.e. leukotriene $B_4$and 5-hydroxyeicosatetraenoic acid) in macrophages upon subsequent activation. While expression of the 5-LOX pathway was not altered, p38MAPK and the downstream MAPKAPK-2that phosphorylates and stimulates 5-LOX, were more susceptible for activation upon precedent coculture with A549cells, and the p38MAPK inhibitor Skepinone-L selectively blocked increased 5-LOX product formation. Also, 15-LOX-derived LM including lipoxin A_4, resolvin D2and D5were elevated after co-culture with A549cells correlating to increased 15-LOX-1protein levels. Vice versa, macrophages increased COX product formation in A549cells accompanied by increased COX-2protein expression.

Conclusions: Conclusively, our data reveal that the communication between macrophages and cancer cells can strikingly modulate the biosynthetic capacities to produce bioactive LM with potential relevance for tumor biology.