LIPOPOLYSACCHARIDE (LPS) STIMULATES NITRIC OXIDE (NO) PRODUCTION AND MDA-MB-231 HUMAN BREAST CANCER CELLS MIGRATION: PARTICIPATION OF RAS AND SRC KINASE

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Ras isoforms (H-, N-, K-) operate as molecular switches regulating pathways associated with cell proliferation, differentiation, and apoptosis. Differences in relative compartmentalization of Ras isoforms may explain the different signaling responses. Furthermore, post-translational modifications of Ras isoforms direct them to the plasma membrane and to endomembranes. Recently we showed that endothelial NO synthase-derived NO and low concentrations of the s-nitrosothiol s-nitrosoglutathione (GSNO) nitrosylate and regulate the activation of the small GTPase Ras. GSNO-mediated activation of Ras occurred at the plasma membrane and at the Golgi apparatus during stimulation of cell proliferation. In another study, Ras activation at the Golgi apparatus has been suggested to play a major role in cell transformation. NO as well plays a major role in tumor progression putatively through s-nitrosylation of Ras and activation of downstream signaling events. In this communication we described our initial findings on LPS-mediated stimulation of endogenous NO production in MDA-MB-231 triple-negative breast cancer cells its relationship with Ras and Src kinase activation, and its consequence on cell migration. Endogenous NO production was estimated by using the cell permeable fluorophore DAF-2DA. MDA-MB-231 cells were incubated for 16 hours with LPS (500 ng/mL). Cell migration was evaluated under the same experimental conditions used for the determination of the intracellular NO production using the in vitro “wound healing assay”. Cells migrate after 16 hours stimulation with LPS and migration was inhibited by pre-incubation of cells with PP2 (Src kinase inhibitor). Inhibition of migration was not observed in cells pre-incubated with FPTIII (Ras inhibitor) followed by stimulation with LPS. In conclusion, endogenous NO generated upon stimulation of MDA-MB-231 breast cancer cells with LPS is associated with cell migration. Activation of Src is essential for cell migration. Cell migration under these conditions is independent of Ras activation.

Key Words: nitric oxide, Lipopolysaccharide, Ras.