INHIBITION OF CCL2 RELEASE AS A NOVEL METHOD TO PREVENT METASTASIS IN TRIPLE NEGATIVE BREAST CANCER

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Abstract: Triple-negative breast cancer (TNBC) is an aggressive breast cancer that disproportionately affects African American women with serious clinical outcomes especially after the metastasis of this disease. Breast cancer metastasis remains the main cause of death and contributes to poor prognosis in the majority of cases. Research efforts have been directed at interfering with key factors important in metastasis as alternative ways for cancer treatment. Breast cancers are circumscribed in a milieu of TNFα secreting cells, which act on the tumor cell TNFα receptors, evoking a sharp rise in the release of chemotactic proteins (e.g. MCP-1 /CCL2). Subsequently, CCL2 directs inward infiltration of tumor-associated macrophages (TAMs) and other chemokines enabling migration of tumor-associated neutrophils (TANs), myeloid-derived suppressor cells, T-regulatory cells, T helper IL-17-producing cells, metastasis-associated macrophages and cancer-associated fibroblasts. These infiltrates collectively suppress host immune capacity to phagocyte cancer cells and allow tumor growth, metastasis, stem cell survival and immune evasion. In the current study, we investigated the potential of apigenin, a known anti-inflammatory flavonoid, to downregulate the first step in this process – being the TNFα mediated release of chemokines from human breast cancer cells (MDA-MB-231 cells). The data obtained show that TNFα evoked a sharp rise in the release of several proteins: granulocyte-macrophage, colony-stimulating factor (GM-CSF), CCL2, IL-1α and IL-6, all effectively attenuated by apigenin. The effects of TNFα ± apigenin on the release of CCL2-and IL-α were validated by antibody array, ELISA, and RT-PCR. The data also demonstrate that the signaling pattern associated with cytokine attenuation by apigenin is primarily through IKBKe inhibition. IKBKe is an inhibitor of the kappa light polypeptide gene enhancer in B-Cells, Kinase Epsilon. The attenuation of IKBKe by apigenin was validated by both (total) mRNA and protein expression. The data obtained show that the attenuation of CCL2 by apigenin in the presence TNFα paralleled the suppression of phosphorylated extracellular signal-regulated kinase 1 (ERK 1, 2). In summary, these findings suggest that TNFα and IL-1 α are equally capable of evoking the elevated release of CCL2 from breast cancer cells, both attenuated by apigenin - through downregulating IKBKe transcription and ERK 1, 2 phosphorylation. The importance of CCL2 in driving tumor malignancy potential in TNBC is well known. The capacity of apigenin to block CCL2 is important because this single event is controlling the infiltrating/migratory activity of the tumor. There is sufficient evidence to show that the initial release of CCL2 marks an irreversible turning point for tumor infiltration and immunological evasion. Natural plant-derived chemicals that can downregulate CCL2 - typically result in impaired migration, less proclivity for metastasis enabling greater efficacy of immunotherapies and chemotherapy drugs. In summary, these findings show that apigenin can block CCL2 in a TNBC cell line. While the experimental evidence for the therapeutic application of apigenin in cancer treatment is growing, human clinical trials are lacking.

Keywords: MCP-1, TNFα, Apigenin, Cytokine, Metastasis

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