Abstract

Melanoma represents a significant and often fatal form of cancer, with metastasis being a primary cause of cancer-related deaths. Immunotherapy aims to stimulate the immune system to eliminate tumors, but even when used together with traditional chemotherapy, still only has <50% success rate. Chemotherapeutic drugs induce tumor cell death, primarily through apoptosis, which is 'silent' and does not incite inflammation or immune cell infiltration into the tumor. During metastasis, tumor cells migrate through confined spaces. CLASP proteins protect organelles and cellular integrity during tumor cell metastasis; when CLASP proteins are depleted, tumor cells migrating through tissue die through a mechanical cell death. The immunological impact of CLASP depletion-induced cell death remains unknown. This study aims to explore whether macrophages, innate immune cells that sense neighboring dying cells, trigger inflammatory cytokine responses specifically to mechanical, as opposed to chemotherapeutic, melanoma cell death. To model mechanical cell death during metastatic migration, CLASP1, a microtubule stabilizing protein, was depleted, and cells were physically compressed to simulate forces encountered during metastasis. Chemotherapy was simulated using a B-Raf inhibitor and the apoptosis inducer staurosporine. Human THP-1 cells, differentiated to a macrophage-like phenotype, were used to represent sentinel macrophages in the tumor surroundings. The study measured macrophage cytokine responses after exposure to melanoma cell supernatants (untreated, mechanically killed, or treated with chemotherapy drugs). To ensure selectivity, doses of staurosporine or the B-Raf inhibitor were tested to kill 1205Lu human melanoma cells without affecting macrophages. Measurements of melanoma and macrophage cell death were conducted using the ATPlite assay at various timepoints and drug doses. Subsequent supernatant transfer experiments assessed macrophage cytokine responses through ELISA or qPCR. Surprisingly, CXCL10 release was specifically observed in macrophages exposed to supernatants from CLASP-depleted compressed 1205Lu melanoma cells, indicating a pro-inflammatory state and recognition by THP-1 macrophages. In contrast, using the B-Raf inhibitor or staurosporine to induce apoptotic cell death did not trigger CXCL10 release. These findings suggest the potential of macrophages to induce CXCL10 release when employing a novel anti-migration approach to treat melanoma in vitro. This approach has the potential to recruit immune cells to the cancer site and activate an immune response and may lead to new combination anti-migrastatic drugs and immunotherapy.

Keywords: mechanical cell death, macrophages, THP-1, CLASP, B-Raf inhibitor, inflammation, cancer, immune response, CXCL10