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 cell death, 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 induce 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. Preliminary observations indicated that CXCL10 levels varied in macrophages exposed to supernatants from different conditions. Notably, macrophages exposed to supernatants from CLASP-depleted compressed 1205Lu melanoma cells appeared to show CXCL10 release. In contrast, supernatants from melanoma cells treated with the B-Raf inhibitor or staurosporine did not show this pattern. These observations suggest a potential difference in macrophage response depending on the method of melanoma cell death. Further investigation with repeated and statistically analyzed

experiments is necessary to validate these findings and fully understand the implications and changes between biological replicates. This approach however might have the potential to recruit immune cells to the cancer site and activate an immune response that could eventually lead to new combination anti-migrastatic drugs used in immunotherapy.

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

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