

PI-18

EXAMINATION OF CYTOTOXIC COMPOUNDS ON DIFFERENT TUMOR CELLS TO CELL VIABILITY AND TO THEIR MIGRATION PROPERTIES WITH A NOVEL CELL-MICROELECTRONIC SENSING TECHNIQUE

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We examined the effects of cytotoxic compounds on the migration and proliferation properties of human glioblastoma, liver carcinoma and melanoma cells with a novel cell microelectronic sensing technique.

Real-Time Cell Analyzer (RTCA) DP is a novel cell migration and invasion assay system that uses the Boyden Chamber principle but does not involve any fixation, labeling or counting of the cells. The core of the system is the CIM-Plate device, composed of an upper chamber and a lower chamber. The upper chamber has 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate or polyester membrane. The median pore size of this membrane is 8 µm. The membrane contains microelectronic sensor arrays that are integrated on its bottom surface. Migration of cells will occur through these electrodes, which changes impedance, and will increase cell index, a unitless parameter, which is calculated as a relative change in actual impedance. The more cells migrate, the higher the cell index will be. RTCA SP is also a microelectronic cell sensor method, where microelectrodes are integrated in the bottom of a microtiter plate (96-well E-plate) and measures adhesion, proliferation or cytotoxicity. One of the most important advantages of the RTCA SP and DP system compared to other cell invasion and migration assays is that this system does not require any labels or reporters, so that the experiment is physiologically more relevant. The real-time measurement can detect changes continuously, which means that the system can give information at any stages of the experiment.

Our results based on the migration experiments were validated by the RTCA SP system. We have tested the real-time effects of numerous molecules in single and in combination treatments. The RT-CES method, when used as described here, is advantageous for pharmaceutical screening of molecular libraries compared to other classical methods.

PI-19

EXAMINATION OF SUBCUTANEOUS B16 MELANOMA CELL-DERIVED PRIMARY TUMOR GROWTH IN STAT6 DEFICIENT MICE

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Tumor tissue contains different immune cells which can generate an immune response against tumor cells. Paradoxically, the developing tumor can often induce a protumoral immune-microenvironment which inhibits anti-tumor immunity while enhancing tumor growth, invasion and vascularization.

CD4⁺ Th1 type T cells and M1 macrophages are essential components of the anti-tumor immune response activating CD8⁺ cytotoxic T cells and reducing tumor growth by tumor cell destruction. In contrast, Th2 cells and M2 macrophages inhibit anti-tumor immunity as well as promote tissue remodeling and tumor progression. However, some in vitro results suggest that M2 macrophages can also inhibit tumor cell proliferation. STAT6 transcription factor has a central role in the development of Th2 cells and M2 macrophages, since it transmits the signal of Th2 type cytokines (IL-4, IL-13) towards the nucleus controlling the expression of hundreds of target genes. Th2 type cytokines can be produced by tumor-infiltrating immune cells and, in some cases, also by tumor cells. In the absence of STAT6 an increased Th1 response, CD8⁺ cytotoxic T cell and M1 macrophage differentiation can be observed.

The main goal of our work was to determine the role of STAT6 transcription factor in tumor development using subcutaneous B16 mouse melanoma tumor model in STAT6 knock-out and wild type mice. Primary tumors were removed 14 days after inoculation and individual tumor weights were measured. We also examined the basal structure and macrophage content of tumors using immunohistochemistry, and characterized the tumor's immunological status with measurement of different immune cell markers by quantitative PCR.

Our results show an increased primary tumor growth in STAT6 deficient mice compared to the wild type animals. Basal structure of the tumor tissue was similar in both groups, but peripheral tumor cell infiltration to the neighboring tissues was enhanced in STAT6 deficient mice. Gene expression analysis of the tumor tissue revealed significant down-regulation of M2 macrophage marker arginase 1 and anti-inflammatory cytokine IL-10 in STAT6 knock-out mice-derived tumors. These results suggest that STAT6 may have an influence on primary tumor growth and have a role in controlling the phenotypic switch of tumor-infiltrating immune cells.