A New Method to Generate Quadromas by Electrofusion and FACS Sorting

Electrofusion of a Weakly Immunogenic Neuroblastoma with Dendritic Cells Produces a Tumor Vaccine

The absence of surface costimulatory molecules explains in part the lack of an effective antitumor immune response in tumor-bearing animals, even though unique tumor antigens may be presented by class I MHC. We determined that the immunogenicity of a murine neuroblastoma, Neuro-2a, which lacks surface costimulatory molecules, could be increased by electrically induced fusion with dendritic cells. Electrofusion induced a higher level of cell fusion than polyethylene glycol, and tumor/dendritic cell heterokaryons expressed high levels of costimulatory molecules. While Neuro-2a was unable to induce the proliferation of syngeneic or allogeneic T cells in vitro, fused cells were able to induce T cell responses both in vitro and in vivo. When fixed dendritic tumor cells were used as a cancer vaccine, immunized mice were significantly protected from challenge with Neuro-2a. We propose that electrofusion with patient-derived tumor and dendritic cells may provide a rapid means to produce patient-specific tumor vaccines.

BTX Electrofusion is as easy as 1 - 2 - 3 !

1. An oscillating AC waveform pulse aligns cells to be fused
2. A DC waveform pulse is applied fused cells
3. A final AC waveform pulse is briefly applied to hold fused cells together during recovery post fusion

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RGS13 Controls G Protein-Coupled Receptor-Evoked Responses of Human Mast Cells

IgE-mediated mast cell degranulation and release of pro-inflammatory mediators induced by allergens elicits allergic responses. Although G protein-coupled receptor (GPCR)-induced signals may amplify IgE-dependent degranulation, how GPCR signaling to mast cells is regulated remains incompletely defined. We investigated the role of regulator of G protein signaling (RGS) proteins in the modulation of these pathways in human mast cells. Several RGS proteins were expressed in mast cells including RGS13, which we previously showed inhibited IgE-mediated mast cell degranulation and ampullositis in mice. To characterize how RGS13 affects GPCR-mediated functions of human mast cells, we analyzed human mast cell lines (HMC-1 and LAD2) derived from RGS13 mice with specific small interfering RNA or short hairpin RNA and HMC-1 cells overexpressing RGS13. Transient RGS13 knockdown in LAD2 cells led to increased degranulation to sphingosine-1-phosphate but not to IgE-Ang or CAs. Relative to control cells, HMC-1 cells stably expressing RGS13-targeted short hairpin RNA had greater Ca2+ mobilization in response to several natural GPCR ligands such as leukotriene C4, thrombin, leukotriene B4, and complement C5a. Relative to control cells, HMC-1 cells overexpressing RGS13 showed inhibited IgE-mediated Ca2+ mobilization, Akt phosphorylation, chemotaxis, and cytokine (IL-8, IL-6) secretion induced by cytokine concomitant in blue with 4- [2,4-diethynylphosphinyl]-2H-pyrimidine.

Abstract—Clinically chemo-refractive types of cancers do not respond well to conventional therapies. To treat and enhance the efficacy of drug delivery for these cancers, we have developed an in vitro model of a combination therapy using adult mesenchymal stem cells. Adult mesenchymal stem cells have been used for this study primarily because of their ability to home towards tumor cells, making the possibility to practice targeted tumor therapy more realistic. These cells, derived from human adult bone marrow were subjected to high intensity, short duration (1200V/cm, 100μs), and low intensity, long duration (200V/cm, 40ms and 450V/cm, 25ms) pulses. The effect of these voltages on the viability and proliferation ability of these cells in the presence and absence of Bleomycin (FDA approved chemotherapeutic used for treating various cancers) indicate the possibility of transfer of this technique to clinical practice for effective electro-molecular targeted stem cell therapy. An analysis of the electrical energy applied vs. the viability illustrates a linear relationship. The dose curve exhibits a non-linear relationship. These results indicate that the high efficacy of MSC-targeted combination therapy would provide efficient, economical, and enhanced clinical benefit for many types of cancers which need alternate treatments.

RNAi screening of the tyrosine kinome identifies targets that do not contain mutations, such as JAK1 and the focal adhesion kinases of an efficient, functional screening assay using RNAi technology to directly assess and compare the effect of individually targeting each member of the tyrosine kinase family. We demonstrate that siRNA screening can identify tyrosine kinase targets containing activating mutations in Janus kinase (JAK)3 (JAK2V617F) in CMK cells and c-Kit (PDGFRα) in HMC-1 cells. In addition, this assay identifies targets that do not contain mutations, such as JAK1 and the focal adhesion kinases (FAK), that are crucial to the survival of the cancer cells. This technique, with additional development, might eventually offer the potential to match specific therapies with individual patients based on a functional assay.

Figure 7: Cancer stem cells (CSCs) are cancer cells found within tumors and are characterized by their ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorogenic (tumor-forming); perhaps in contrast to other non-tumorogenic cancer cells.