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P144

Evaluating CAR-T cells efficiency against solid tumors models

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Chimeric antigen receptors (CARs) have revolutionized T cell-based immunotherapy for the treatment of cancer. Due to success of CAR-T therapy in oncohematology its potential use against solid tumors is now being actively studied. Key issues to consider in such studies include potentially low specificity and efficacy of CAR-T against malignant tumor cells and the need to target specific antigens. The basic structure of CAR consists of an extracellular region linked to a hinge region responsible for flexibility, trans-membrane region and intracellular signaling domains. In current study we evaluated anti-CD19 CAR-T cells against several 3D bioprinted solid tumor models. We constructed plasmid with 2nd-generation anti-CD19 CAR and also recombinant vector containing CD19 gene under control of internal ubiquitin C promoter and puromycin resistance gene. T cells obtained from healthy donor were activated and transduced with lentivirus. CD19-positive cells were generated by transduction of H522 solid tumor cell lines with CD19_p2a_PuroR recombinant lentiviral vector. After that anti-CD19 CAR-T cells were applied onto CD19-positive tumor cell 3D constructs bioprinted using hydrogel composition. Efficacy of anti-CD19 CAR-T cells was assessed using viability assay and confocal microscopy. According to the results, anti-CD19 CAR-Ts were efficient against CD19-positive cancer cells in 2D monolayer cell cultures and 3D bioprinted solid tumor models. Work supported by RSF grant 19-74-20026.

P145

AML-derived extracellular vesicles transmit immunomodulatory potential

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Trophic factors including extracellular vesicles (EVs) secreted by AML cells have recently been described as potent modulators instructing the leukemic niche. We observed that AML-derived EVs but not the donor cells and not their secreted/soluble factors (sol.F.) spread an immunomodulatory capacity capable of inhibiting T cell proliferation. AML cell lines (HL-60, OCI-AML3, MOLM-14, KG-1) were cultured under static vs. dynamic (2D vs. 3D) conditions, in ambient air as compared to organotypic reduced oxygen environment in defined particle-depleted media. Tunable resistive pulse sensing indicated a mean particle release ranging from 2x108-2x109 per ml/48h with a mean diameter of app. 150 nm (range: 50 – 750 nm). Large scale crude EV propagation was done by tangential flow filtration (TFF) to obtain >100x particle enrichment. An additional TFF purification step was introduced to separate sol.F. from AML-EVs, followed by size exclusion chromatography (SEC) or ultracentrifugation (UCF) to obtain virtually pure EVs (protein <1.0 mg/mL with UCF and <0.5 mg/ml with SEC). AML-EV morphology was confirmed by electron microscopy and identity by immunoblotting and flow cytometry. Bead-based EV surface profiling showed hematopoietic and EV-specific markers. Single EV flow cytometry revealed calcein-positive events indicating the presence of intact EVs in our preparation. We further observed that 4/4 AML-EV preparations but only 1/4 AML cell lines and 0/4 secreted factor fractions inhibited T cell mitogenesis. Additional functional tests are currently underway. These data show that AML-EVs but not the parental cells or leukemia-derived sol.F. display a previously unexpected immunomodulatory capacity indicating novel targets for therapeutic intervention.

P146

The effect of co-culturing of MSC and SH-SY5Y and cisplatin treatment on proliferative activity and caveolin-1 mRNA expression

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