Modeling antitumor responses with spheroid–immune cell cocultures

Cocultures of human colorectal tumor spheroids with immune cells reveal the therapeutic potential of MICA/B and NKG2A targeting for cancer treatment

Courau T, Bonnereau J, Chicoteau J, Bottois H, Remark R, Assante Miranda L, Toubert A, Blery M, Aparicio T, Allez M, Le Bourhis L (2019) *J Immunother Cancer* 7:74.

Researchers have yet to find effective immunotherapies to treat patients afflicted with colorectal cancer, in part due to the lack of accessible models for identifying key effectors and inhibitors that might serve as appropriate antibody targets. In their recent publication, Le Bourhis and his team at INSERM in Paris describe the preparation of colorectal cancer tumor spheroids and their coculture with allogenic immune cells, including T cells and NK cells, that rapidly infiltrate the cell line–derived spheroids. They demonstrate the use of these cocultures to study antitumor immune responses such as tumor cell apoptosis and spheroid destruction *in vitro*, and further confirm their observations *ex vivo*.

By producing individual, uniform spheroids in matrix- and growth factor–free medium, these researchers were able to precisely and reproducibly manipulate coculture settings, as well as monitor immune cell infiltration, measure spheroid volume, and assess tumor cell apoptosis by flow cytometry and fluorescence imaging (Figure 1). Their characterization of the infiltrating (IN) and non-infiltrating (OUT) immune cells showed that memory T cells and NK cells induce tumor cell destruction via interferon and NKG2D-MICA/B pathways.

Based on these observations, this team evaluated antibodies specific to NKG2D, an activator of cytotoxic responses, and its ligands MICA and MICB. When they treated cocultures with anti-MICA/B antibody, spheroid destruction by NK cell infiltration and activation was enhanced. They also observed the upregulation of HLA-E (a ligand of the inhibitory NKG2A receptor) on tumor cells and an increase in NKG2A expression on infiltrating NK cells, leading to an assessment of the effects of anti-NKG2A antibody. Although treatment of cocultures with anti-NKG2A antibody alone did not show a significant response, the combined treatment with anti-MICA/B antibodies provided a coordinated, synergistic antitumor effect, implicating both the NKG2D-MICA/B and NKG2A–HLA-E pathways in immune-mediated tumor disruption. These observations were confirmed using spheroids derived directly from primary tumors of colorectal cancer patients in conjunction with autologous tumor-infiltrating lymphocytes. This work illustrates the utility of tumor spheroids as a relevant and representative model for defining the interactions between tumor cells and lymphocytes in the effort to identify critically needed cancer immunotherapies. ■

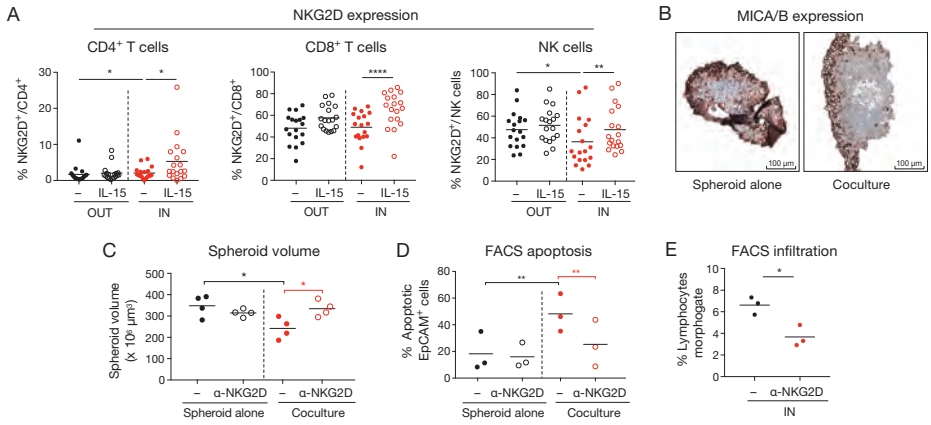

Figure 1. NKG2D-MICA/B pathway is engaged in cocultures. (A) NKG2D expression by CD4 T cells, CD8 T cells, and NK cells in the IN (infiltrating cells isolated from sedimented, rinsed, trypsinized spheroids) and OUT (non-infiltrating cells) compartments, in the presence or not of IL-15, at 24 hr as measured using the Invitrogen™ Attune™ NxT Flow Cytometer; n = 18 independent experiments. **(B)** MICA/B expression by tumor cells in the spheroids cocultured or not with CD19⁻CD14⁻ PBMCs, as measured by immunohistochemistry at 24 hr; representative images of 1 experiment, acquired using the Invitrogen™ EVOS™ FL Cell Imaging System. Analyses of **(C)** spheroid volume, **(D)** tumor cell apoptosis, using Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent (Cat. No. C10423), and **(E)** spheroid infiltration 48 hr after coculturing HT29 spheroids with CD19⁻CD14⁻ PBMCs in the presence or not of anti-NKG2D blocking antibodies; n = 3–4 independent experiments. Statistical significance of A and C–E were analyzed using the Wilcoxon matched-pairs signed rank test and the paired *t* test, respectively (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$, **** $p < 0.0001$). Reproduced with permission from Courau T et al. (2019) *J Immunother Cancer* 7:74, and under the Creative Commons Attribution 4.0 International License (creativecommons.org/license/by/4.0/).