

Supplemental Materials and Methods

Cancer-associated CD43 glycoforms as target of immunotherapy.

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Cell-cycle and apoptosis analysis

For monoparametric cell-cycle analysis, cells were washed in PBS and fixed with cold 70% ethanol for a minimum of 24 h at 4°C. Then, 2×10^6 cells were washed twice in PBS and incubated with 2ml of 20µg/ml propidium iodide (Sigma-Aldrich, St Louis, MO, USA) in PBS containing 0.002% Nonidet P40 (Roche Applied Science, Penzberg, Germany) and 12.5µg/ml RNase A (Sigma-Aldrich) at RT for a minimum of 60 min. For each sample, at least 20,000 cells were analyzed for DNA content using a FACSCalibur flow cytometer (BD Biosciences, Erembodegem, Belgium). The DNA histogram was analyzed for cell-cycle progression using ModFit LT v3.1 software (Verity Software House, Topsham, ME, USA).

Surface Plasmon Resonance

Surface plasmon resonance (SPR) was performed using Biacore 3000 optical biosensor equipped with research-grade CM5 sensor chips (Biacore GE Healthcare). Data were collected at 2.5 Hz. The UN1 mAb (100 µg/mL) was resuspended in 10 mM sodium acetate, pH 5.0, and immobilized on individual sensor chip surfaces at a flow rate of 5 µL/min using standard amine-coupling protocols to obtain densities of 8-12 kRU. Peptides concentration series were prepared as two-fold dilutions into running buffer. For each sample, the binding analysis was performed using a 5 points concentration series, 100 nM, 250 nM, 500 nM, 1 µM and 2.5 µM;

triplicate aliquots of each compound concentration were dispensed into single-use vials, capped, and randomized in the instrument's autosampler rack. Multiple blank samples of running buffer alone were included in each analysis. Five of these blanks were analyzed at the beginning of the analysis and the remaining blanks were interspersed throughout the analysis for double-referencing purposes. Binding experiments were performed at 25°C, using a flow rate of 50 µL/min, with 60 s monitoring of association and 200 s monitoring of dissociation. Regeneration of the surfaces was performed by a 140 s injection of 50 mM glycine pH 9.5, followed by an ExtraClean wash command that automatically flushes the sample delivery system with running buffer. Sensorgrams were set to zero on both the response and time axes at the beginning of each injection and double referenced. Hallmarks that make this data set a good one include responses that are concentration dependent, replicate injections that overlay, and clearly discernible exponential curvature during both the association and dissociation phases. Simple interactions were adequately fit to a single-site bimolecular interaction model (A+B=AB), yielding a single K_D . Sensorgram elaborations were performed using the BIAevaluation software provided by Biacore (GE Healthcare).

Immunohistochemistry

Four quadrantectomy for infiltrating ductal carcinoma and four partial gastrectomy to adenocarcinoma (differentiated infiltrating adenocarcinomas) were used. Immunohistochemical analysis was performed as previously described (6, 7). Sections of 6 µm thick were obtained from paraffin blocks and mounted on glass slides. Before staining, endogenous peroxidase activity was quenched for 15 minutes incubation of the section with 0.3% hydrogen peroxide. Slides were washed twice with PBS, incubated with normal horse serum following by overnight incubation with the UN1 mAb (0.33 µg/mL), control IgG (2.0 µg/mL), IgG purified from phage 2/165-immunized mice (2/165 IgGs) or wild type phage-immunized mice (wt IgGs). Subsequently, slides were rinsed and stained by peroxidase-antiperoxidase staining method (6, 7). The stain was developed using diaminobenzidine and the samples were analysed with the Leitz Orthoplan microscope (Wetzlar, Germany) equipped for microphotography. For competition assays, the UN1 mAb or 2/165 IgGs were pre-incubated overnight at 4°C with 2/165 phage or wt phage (2.5×10^{13} particles/mL), as well as G23 or scrambled peptide (500 µg/ml), and then incubated to tumor tissue sections.