Supplemental figure legends

Supplemental figure 1

GFP-labeled SCC9 cells could be found in donor site, depletion of NK cells in Rag1$^{-/-}$ mice and purity of human NK cells isolated from healthy individuals. Similar levels of FN protein were observed in SCC9-parental and SCC9-seeded cells. N-cadherin/ KLRG1 axis expression on mouse CTCs and NK cells. (A) Contralateral seeding experiments were performed using GFP-labeled SCC9 cells as donor cells and RFP-labeled SCC9 cells as recipient cells. Self-seeding was detected by fluorescence microscopy 60 days after inoculation. Bar: 500 μm. (B) Depletion of NK cells in Rag1$^{-/-}$ mice was verified by flow cytometry analysis. (C) Purity of fresh human NK cells isolated from healthy individuals was confirmed by flow cytometry analysis. (D) No significant difference in the expression of FN was found between SCC9-parental and SCC9-seeded cells. Silencing of FN did not affect susceptibility of the tumor cells to NK-cell killing and supernatants from FN-silenced SCC9-seeded cells showed no effect on the cytotoxicity of NK cells. (E) Detection of N-cadherin in CTCs harvested from the contralateral seeding model. From left to right: CK staining; N-cadherin staining; DAPI staining; merged image. Bars: 50 μm. (F) Expression of KLRG1 on mouse NK cells was detected by FCAS analysis. Data represent 3 independent experiments done in triplicate (*in vitro* model) or with 6 mice per group (*in vivo* model) (mean ± SD). ns, no significance, two-way ANOVA analysis (D). RFP, red fluorescent protein; GFP, green fluorescent protein; NK, natural killer; NC, negative control. si, small interfering RNA; CK, cytokeratin; N-cad, N-cadherin; DAPI, 4‘,6-diamidino-2-phenylindole.

Supplemental figure 2

Validation of N-cadherin knockdown or overexpression in SCC9-parental and SCC9-seeded cells and purity of isolated KLRG1 (+) and KLRG1 (-) NK cells. (A) Testing and validation of small interfering RNA against human N-cadherin, siRNA 2 appeared most effectively at reducing N-cadherin expression in SCC9-parental and SCC9-seeded cells and was selected to produce shN-cad lentiviral particles. (B) SCC9-parental cells were infected by N-cadherin overexpressing (oeN-cad) lentivirus. SCC9-seeded cells were infected with short hairpin N-cadherin (shN-cad) lentivirus. Protein levels of N-cadherin in SCC9-parental cells, SCC9-seeded cells, SCC9 oeN-cad cells and SCC9-seeded shN-cad cells were tested by Western blot. (C) The separation efficiency of isolated KLRG1 (+)/(-) NK subsets were confirmed by flow cytometry analysis. Data represent 3 independent experiments done in triplicate (A, B) or with 10 healthy individuals (C) (mean ± SD). si, small interfering RNA; shN-cad, short hairpin N-cadherin; oeN-cad, overexpress N-cadherin.

Supplemental figure 3

Representative FACS histograms of cytolytic effector molecules (left), four NKARs (middle) and NKIRs (right) expression in NK-cell populations following indicated treatments shown in figure 4A. Gray histograms represent the isotype controls and the coloured histograms represent the markers of interest. Data represent 3 independent experiments done in triplicate. DMEM, dulbecco‘s modified eagle medium; shNC, short hairpin negative control; shN-cad, short hairpin N-cadherin; NK, natural killer; FACS, fluorescence activated cell sorting; N-cad Ab: N-cadherin blocking antibody; rhN-cad: recombinant human soluble N-cadherin; NKARs, NK cell activation receptors; NKIRs, NK cell inhibitory receptors.

Supplemental figure 4

Protein and mRNA expression of ADAM10 in tumor cells under various treatment conditions and its biological effects. (A) The expression levels of six individual variants of ADAM10 were quantified by qRT-PCR. Similar levels of mRNA transcript corresponding to full-length ADAM10 with proteolytic activity (red box, ENST00000260408.8) were observed in SCC9-parental and SCC9-seeded cells. (B) Testing and validation of siRNA against human ADAM10, siRNA 1 was selected to produce shADAM10 lentiviral particles. siRNA, small interfering RNA. (C, D) Protein (C) and mRNA (D) expression of ADAM10 in tumor cells after exposure to NK cell supernatants or cytokines treatment. (E) ADAM10 expression in cells treated with cytokines, the translation inhibitor cycloheximide (CHX) or the transcription inhibitor actinomycin D (ActD). (F) NK cell cytotoxicity against K562 cells with the indicated treatments at E/T ratios of 5:1, 10:1 and 20:1. (G) ADAM10 mRNA levels in SCC9-seeded cells received fluid flow and the Piezo1 inhibitor GsMTx4 treatment. (H) Piezo1 expression in SCC9-seeded cells received fluid flow treatment. (I) The time course for the change in ADAM10 expression after removing IFN-γ/TNF-α or shear stress treatment. (I) NK cell cytotoxicity against K562 cells with the indicated treatments at E/T ratios of 5:1, 10:1 and 20:1. Data represent 3 independent experiments done in triplicate. **P*< 0.05; ***P*< 0.01; ns, no significance; unpaired Student's *t* test (A); two-way ANOVA analysis (F, J); one-way ANOVA and Tukey–Kramer multiple comparison tests (D, G).

Supplemental figure 5

NK challenging cannot cause SCC9-parental cells to acquire a SCC9-seeded cell like phenotype. (A) NK challenging did not lead to upregulation of N-cadherin expression in both SCC9-parental and SCC9-seeded cells. (B) SCC9-parental cells pre-exposed to NK cell supernatants displayed a similar susceptibility to NK-mediated lysis as the untreated SCC9-parental cells, and their

supernatants showed a weak inhibitory effect on the cytotoxic activity of NK cells when compared with SCC9-seeded cells. (C) Microscopic images of the tissue regions after microdissection. Square tissues were dissected from the SCC9-parental cell enriched sample region. Irregular tissues were dissected from the SCC9-seeded cell enriched sample region. Data represent 3 independent experiments done in triplicate. ***P*< 0.01; ****P*< 0.001; ns, no significance; two-way ANOVA analysis (B).

Supplemental figure 6

Control of each group in Fig. 6. (A, B) Seeded tumors were visualized by fluorescence microscopy at 60 days after inoculation using contralateral seeding model, SCC9-seeded cells were inoculated as donor cells. Bars: 500 μm. (C, D) Seeded tumors were visualized by fluorescence microscopy at 60 days after inoculation using contralateral seeding model, SCC9-seeded shN-Cad cells (C) and SCC9-seeded shADAM10 cells (D) were inoculated as donor cells. Bars: 500 μm. (E, F) Seeded tumors were visualized by fluorescence microscopy at 60 days (E) or 30 days (F) after inoculation using contralateral seeding model, SCC9 RFP cells were inoculated as donor cells. Bars: 500 μm. Data represent two independent experiments with 6 mice per group. RFP, red fluorescent protein; GFP, green fluorescent protein; shN-cad, short hairpin N-cadherin; shADAM10, short hairpin ADAM10.

Supplemental figure 7

IVFC was used to assess the phenotype of NK cells isolated from the spleen of mice in each experimental group. (A) *In vivo* flow cytometry (IVFC) was used to detected CTCs in mouse models five hours after tail vein injection with tumor cells. The mouse was anesthetized and placed on a

heated stage. Vein, mouse ear vein; artery, mouse ear artery; laser slit, laser light focused across the artery. (B) Representative FACS histograms of cytolytic effector molecules, NKARs, NKIRs expression in NK-cell populations isolated from the spleen of mice shown in figure 7J. Gray histograms represent the isotype controls and the coloured histograms represent the markers of interest. Data represent 3 independent experiments done in triplicate with 5 mice per group. NKARs, NK cell activation receptors; NKIRs, NK cell inhibitory receptors.

Supplemental figure 8

Schematic representation of membrane-bound or soluble N-cadherin triggering NK cell functional exhaustion and protecting CTCs from NK cell-mediated killing via interacting with the KLRG1 receptor.

Supplemental figure 9

Gating strategy for the FACS based immune profiling experiments. (A) Gating strategy for figure 1C. (B) Gating strategy for figure 2I and supplemental figure 1C. (C) Gating strategy for figure 4A, B, figure 7J, supplemental figure 2C, supplemental figure 3 and supplemental figure 7B. (D) Gating strategy for supplemental figure 1B. (E) Gating strategy for supplemental figure 1F.