Supplementary Figures for

MicroRNAs Reprogram Normal Fibroblasts into Cancer Associated Fibroblasts in Ovarian Cancer

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**Fig. S1: Invasion of the patient-derived fibroblasts.**

A, Schematic of the dynamic in vitro invasion assay monitoring invasion of normal omental fibroblasts (NOFs), adjacent NOFs or cancer-associated fibroblasts (CAFs) labeled with CMFDA (Cell Tracker Green). Invasion through matrigel was tracked by time lapse 3D confocal microscopy.

B, Patient-derived CAFs invade faster than NOFs or adjacent NOFs. Left, invasion of the indicated primary fibroblasts. Right, Quantification of the speed of invasion in the experiment shown on the left.
**Fig. S2: Characterization of CAFs and induced CAFs.**

**A**, Top, schematic of an *in vitro* assay to follow invasiveness of ovarian cancer cells co-incubated with either NOFs, adjacent NOFs, or CAFs in real time using time lapse 3D confocal microscopy. Bottom left, invasion of three OvCa cell lines co-incubated with the indicated patient-derived fibroblasts (NOFs, aNOFs, or CAFs). Fluorescently labeled cancer cells are in red, fluorescently labeled fibroblasts are green. Invasion through matrigel was tracked by time lapse 3D confocal microscopy. Bottom right, quantification of the speed of invasion in assays shown on the left.

**B**, Invasion of HeyA8 cells co-incubated with NOFs (top) or induced (i)CAFs generated through seven days co-culture with HeyA8 OvCa cells (bottom). The speed of invasion was quantified. Values represent mean ± s. d. Significance was calculated using Student’s t-test.
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A

ex vivo

in vitro

Relative expression

aNOF CAF

NOF Induced CAF

p=0.02

p=0.05

p=0.06

p=0.04

p=0.03

p=0.02

B

H&E

scr

miR-214

Adjacent

Tumor

H&E

scr

miR-155

Adjacent

Tumor

F

T

F

T

F

T

F

T

F

T

F

T

H&E

scr

miR-214

Adjacent

Tumor

F

T

F

T

F

T

F

T

F

T

F

T
Fig. S3: Validation of miRNA expression changes in CAFs and induced CAFs identified in the miRNA array.

A, RNA was extracted from CAFs isolated from the omental metastasis of patients with high grade serous OvCa (ex vivo) and matching adjacent NOFs or from NOFs and induced CAFs derived from a two day co-culture of NOFs with HeyA8 cells (in vitro). The indicated miRNAs were quantified by quantitative real time PCR. Changes for corresponding pairs of fibroblasts for each individual patient are shown. Significance was determined using one-tailed paired t-test.

B, In situ hybridization of human ovarian cancer omental metastasis and adjacent normal omentum for miR-214 (top) and miR-155 (bottom). Scale bar = 20 µm. Stippled lines indicate the borders between areas of adipocytes (A) and fibroblasts (F) (rows 1 and 3) or tumor tissue (T) and fibroblasts (F) (rows 2 and 4).
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Fig. S4: Validation of miRNA-transfection in NOFs and CAFs by quantitative real time PCR.

A, Quantification of miRNA expression using real-time PCR in NOFs 48h after transfection with either scrambled control oligonucleotides (ratio scr/LNA scr 1:2) or a combination of miRNA inhibitors for miR-31 and 214 and pre-miR-155.

B, Quantification of miRNA expression using real-time PCR in CAFs 48h after transfection with either scrambled control oligonucleotides (ratio scr/LNA scr 2:1) or a combination of miRNA inhibitor for miR-155 and pre-miR-214 and 31.
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Fig. S5. Altering individual miRNA expression contributes to functional effects of miR-CAF.

A-C, Reprogramming of CAFs to NOFs. Overexpression of miR-214 (214), miRNA-31 (31), or inhibition of miR-155 (LNA anti-miR-155) in CAFs inhibits migration (A), their ability to enhance HeyA8 cell invasion (B), and anchorage independent growth tested by colony formation (C). Negative controls (scr) are indicated.

D-F, Reprogramming of NOFs to CAFs. Inhibition of miR-214 or miR-31, or overexpression of miR-155 (155) in NOF increases their migration (D), their ability to enhance HeyA8 cell invasion (E), and anchorage independent growth (F).

Values represent mean ± s.d. from experiments performed at least three times. Significance was determined using one tailed t-test.
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Fig. S6: The miR-214 seed match is the most highly conserved region in the CCL5 3'-UTR of the human, mouse, and rat gene.

Alignment of the 3'-UTRs of human, mouse, and rat CCL5. Identical nucleotide positions are marked by a red column. The positions of the seed matches of miR-214 and miR-31 are labeled. The 3'-UTR of human CCL5 was truncated. Alignment of the 3'-UTRs revealed that the region of highest identity (10 nucleotides long) contains the seed match for miR-214.
Fig. S7: Orthotropic mouse xenografts.
A, Representative bioluminescence images of mice (day 15) from Figure 4A and B, from Figure 4B. Color scale is in radiance (p/sec/cm²/sr). The entire tumor for each mouse was quantified.
C, Representative Ki-67 staining of tumors from Fig 4A (left), and quantification of Ki-67 staining in tumors from 4 mice/group (right).
D, Representative Ki-67 staining of tumors from Fig 4B (left), and quantification of Ki-67 staining in tumors from 4 mice/group (right). The significance was determined using one tailed t-test.
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Fig. S8: Expression of CCR1 and CCL5.

A, Expression of CCR1 in HeyA8 and SKOV3ip1 ovarian cancer cells. Total RNA from HeyA8, Skov3ip1 and differentiated THP-1 (positive control) cells was converted into cDNA followed by PCR for CCR1 or GAPDH. The amplified products were resolved on a 1% agarose gel and stained with ethidium bromide. Representative image of two independent experiments is shown.

B, Quantitative RT-PCR for human CCL5 in NOFs transfected with either human CCL5 vector or with vector control. Error bars represent triplicate experiments.
Fig. S9: Characterization of the patient-derived fibroblasts.
A, Phase contrast images of primary human normal omental fibroblasts (NOFs), adjacent NOFs (aNOFs) and cancer-associated fibroblasts (CAFs).
B, Immunofluorescence staining (green) of vimentin, \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) and cytokeratin in NOFs, adjacent NOFs and CAFs. All cells were counterstained with DAPI (blue).
C, Immunoblotting for \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) in CAFs and adjacent NOFs (aNOFs) in three different patients. \(\beta\)-actin (actin) was used as a loading control.