Supplemental Note

Immunohistochemical analysis of tumor biopsy tissue

Method. Immunohistochemistry was performed on frozen or paraffin embedded sections of rectal tumor biopsies to establish the pattern of expression of CXCR4, SDF1α and NRP1. Briefly, the sections were air-dried (if frozen) or deparaffinised (if specimens were embedded in paraffin), rehydrated with phosphate buffered saline (PBS) and preincubated with hydrogen peroxide to block endogenous peroxidase activity. The sections were then pretreated with 5% normal donkey or horse serum in PBS, and stained with primary antibody. Subsequently the sections were incubated with the secondary antibody, and the Avidin Biotin complex formed was visualized with 3, 3'-diaminobenzidine tetra hydrochloride. Finally the sections were counterstained with hematoxylin.

The anti-CXCR4 antibody was used in a Dilution of 1:1500 (R&D Systems, Mab 172, Santa Cruz CA, secondary antibody used was Dako anti mouse envision kit), anti-SDF1α antibody was used in a Dilution of 1:40 (Biovision 5388-100, CA, secondary antibody used was Dako anti rabbit envision kit) and anti-NRP1 antibody was used in a Dilution of 1:40, Chemicon AB 9600, secondary antibody used was Dako anti rabbit envision kit).

The slides were examined in a blinded fashion. Three arbitrary 40X fields with tumor were examined in each biopsy. The number of cells positive for the antibody was calculated as a proportion of the number of tumor cells in each field. Internal controls for the stains used were adjoining non-neoplastic colonic epithelia, endothelium, and lymphocytes. Staining of cells was graded as nuclear, cytoplasmic, membrane, or both. The stain was considered strongly positive if the expression pattern was equal or stronger than internal controls and weakly positive if the expression pattern was weaker than internal controls.

Results. Most of the biopsies, before and after treatment, showed positivity for all three markers in tumor cells. There were 89.7% cases positive for CXCR4, with predominantly cytoplasmic or membrane staining (83.3%). Occasional cases showed both nuclear and cytoplasmic staining (**Supplemental Figure S1A,B**). The tumor cells showed SDF1α positivity in 91.1% cases, with 50% cases displaying cytoplasmic or membrane staining (**Supplemental Figure S1C,D**). NRP1 positive tumor cells were seen in 86.5% of cases, with predominantly a cytoplasmic pattern in 82.5% cases (**Supplemental Figure 1E,F**).

Supplementary Figure Legend

Figure S1: Pattern of expression of SDF1α, CXCR4 and NRP1 in rectal cancer biopsies. A. Pretreatment tumor biopsy showing strong cytoplasmic and nuclear staining for CXCR4 in cancer cells (40X). Normal colonic epithelium and lymphocytes are positive internal controls. **B.** Tumor biopsy at day 12 after bevacizumab treatment showing strong cytoplasmic and focal nuclear positivity for CXCR4 in cancer cells. In addition, there is focal staining for CXCR4 in macrophages in the tumor stroma (40X). **C.** Pretreatment tumor biopsy displaying strong SDF1α membrane staining seen in cancer cells and normal colonic epithelium (40X). **D.** Strong membrane staining for SDF1α seen in cancer cells in tumor biopsy obtained at day 12 after bevacizumab treatment. **E.** Pretreatment tumor biopsy showing diffuse nuclear and cytoplasmic staining for NRP1 in cancer cells (40X). **F.** Cytoplasmic staining for NRP1 in cancer cells seen in biopsy obtained at day 12 after bevacizumab treatment.

Figure S2. Representative immunostaining of rectal cancer biopsies and metastatic lesion in the lung. A, VEGFR2 protein expression was detectable only in endothelial cells. **B**, SDF1α expression in lung metastases in a rectal cancer patient after completion of bevacizumab and chemoradiation treatment. (scale bar=100μm; in inset, scale bar=50μm.)