Interleukin-8 and its receptor CXCR2 in the tumour microenvironment promote colon cancer growth, progression and metastasis

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BACKGROUND: Colorectal cancer (CRC) is a leading cause of death in the United States. Increased level of interleukin-8 (IL-8) and CXCR2 on tumours and in the tumour microenvironment has been associated with CRC growth, progression and recurrence in patients. Here, we aimed to evaluate the effects of tissue microenvironment-encoded IL-8 and CXCR2 on colon cancer progression and metastasis.

METHODS: A novel immunodeficient, skin-specific IL-8-expressing transgenic model was generated to evaluate colon cancer growth and metastasis. Syngeneic mouse colon cancer cells were grafted in CXCR2 knockout (KO) mice to study the contribution of CXCR2 in the microenvironment to cancer growth.

RESULTS: Elevated levels of IL-8 in the serum and tumour microenvironment profoundly enhanced the growth of human and mouse colon cancer cells with increased peri-tumoural angiogenesis, and also promoted the extravasation of the cancer cells into the lung and liver. The tumour growth was inhibited in CXCR2 KO mice with significantly reduced tumour angiogenesis and increased tumour necrosis.

CONCLUSION: Increased expression of IL-8 in the tumour microenvironment enhanced colon cancer growth and metastasis. Moreover, the absence of its receptor CXCR2 in the tumour microenvironment prevented colon cancer cell growth. Together, our study demonstrates the critical roles of the tumour microenvironment-encoded IL-8/CXCR2 in colon cancer pathogenesis, validating the pathway as an important therapeutic target.

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Colorectal cancer (CRC) is the third most common cancer and also the leading cause of death from gastrointestinal malignancy in the United States. Colorectal cancer ranks second after lung cancer in death rates in the United States (Siegel et al, 2011). Studies suggest that chemokines and their receptors serve as important regulators of various metastatic and advanced cancers (Coussens and Werb, 2001; Coussens and Werb, 2002). Among them, interleukin-8 (IL-8) and its receptor CXCR2 are two of the most significantly upregulated chemokines in colon cancer (Chen et al, 2004). The role of IL-8 and CXCR2 in tumour development and progression has been well documented in a wide range cancer cells (Brat et al, 2005; Singh et al, 2006; Araki et al, 2007; Yao et al, 2007; Merritt et al, 2008; Waugh and Wilson, 2008; Singh et al, 2009). Expression and secretion of IL-8 by tumour cells enhance proliferation and survival of the cells through autocrine activation, and promote angiogenesis and neutrophil infiltration into the tumour. The proangiogenic effects of IL-8 have been shown to be independent from its chemotactic activity for neutrophils and other pro-inflammatory effects (Strieter et al, 1992; Hu et al, 1993; Li et al, 2003). The IL-8/CXCR2-mediated autocrine properties have been demonstrated to activate the intrinsic mechanism of tumour cells to evade stress-induced apoptosis (Maxwell et al, 2007). Therefore, tumour cell-derived IL-8 can exert profound effects on tumour growth, survival, invasion, angiogenesis, metastases, resistance and recurrence. Nonetheless, studies on the effects of tumour microenvironment-encoded IL-8 and CXCR2 are rather limited, mostly due to the intuitive speculation for the equivalent roles regardless of their sources.

Importantly, a common single-nucleotide polymorphism (SNP) in IL-8 at –251 bp (251-bp upstream of the IL-8 transcription start site) has been associated with increased plasma levels of IL-8 (Hull et al, 2000) and with IL-8 promoter activities (Lee et al, 2005; Ohyauchi et al, 2005). This allele has recently been correlated with an increased risk of developing breast, prostate and gastric cancers (McCarron et al, 2002; Taguchi et al, 2005; Snoussi et al, 2006). We – for the first time – demonstrated that this IL-8T – 251A
polymorphism was individually associated with risk of tumour recurrence (Gordon et al., 2006). Moreover, our classification and regression tree analyses of CRC produced a classification tree with node status and four marker genes (IL-8, intracellular adhesion molecule-1, transforming growth factor-β and fibroblast growth factor receptor-4) (Gordon et al., 2006). Our genotyping study of 12 biomarker genes in 125 patients with advanced CRC found that patients with vascular endothelial growth factor (VEGF) +936 C/C and IL-8 – 251A/A genotype were at the greatest risk to develop tumour recurrence (Lurje et al., 2008), suggesting that polymorphisms in VEGF and IL-8 may serve as a key biomarker to identify stage III CRC patients who are at a great risk for tumour recurrence. In a separate study (Schultheis et al., 2008), we identified statistically significant four polymorphisms in CXCR2 and VEGF and demonstrated that the IL-8A – 251T polymorphism may be a molecular predictor of response to anti-VEGF chemotherapy. The CXCR2 C + 785T and VEGF C + 936T SNPs can be used as molecular markers for progression-free survival (PFS) in cancer patients. Together, the polymorphisms and expression of IL-8 and CXCR2 are strongly associated with not only tumour progression, but also tumour recurrence and oxaliplatin chemoresistance in patients with various cancers, including CRC (Gordon et al., 2006; Lurje et al., 2008; Schultheis et al., 2008; Zhang et al., 2009).

We have recently reported that IL-8 regulates proliferation, migration, angiogenesis and chemosensitivity in vitro and in vivo in colon cancer models (Ning et al., 2011). In addition, our clinical data showed that patients with stage IV CRC display more than 10 times higher serum level of IL-8 (1089 pg ml⁻¹ ± 311), compared with individuals with no evidence of disease (79 pg ml⁻¹ ± 56) (Ning et al., 2011). In the current study, we tested whether the in-vivo tissue microenvironment with either elevated IL-8 or CXCR2 deletion will promote colon cancer growth, invasion and metastases using a novel IL-8 transgenic mouse model and a CXCR2 knockout (KO) model. Our findings using these mouse models demonstrate the critical roles of IL-8/CXCR2 in the tumour environment, which is highly cohesive with our numerous previous experimental and clinical data, and further indicates that IL-8 and CXCR2 may be an important therapeutic target against colon cancer.

MATERIALS AND METHODS

Cell lines and animals

A mouse colon carcinoma cell line CT26 (BALB/c background) and a human colon cancer cell line HCT116 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in RPMI plus 10% calf serum and DMEM plus 10% calf serum, respectively. HCT116 cells stably expressing the firefly luciferase and red fluorescent protein (RFP), named HCT116LR, were generated by transducing HCT116 with a lentivirus co-expressing the luciferase and RFP (Amsbio, Lake Forest, CA, USA), and the transduced cells were pooled and cultured for further studies. All animal studies were approved by the University of Southern California Institutional Animal Care and Use Committee. The keratin-14-based hIL-8 transgenic mouse line (K14-hIL-8) was generated by the USC Transgenic Animal Core by pronuclear injection of a keratin-14-promoter-driven hIL-8 expression cassette, which was made by cloning a PCR-amplified 0.3-kb hIL-8 open reading frame fragment into a BamHI of the keratin-14 expression vector (Vassar et al., 1989) (kindly provided by Dr Elaine Fuchs, The Rockefeller University). The founders, with B6D2 background, were screened for the expression of hIL-8 in their serum and one founder was chosen to establish a line. To transfer the K14-hIL-8 transgene to an immunodeficient mouse background, K14-hIL-8 mice were bred with athymic nude mice (Crl:NU-Foxn1nu) from Charles River Laboratories International, Inc. (Wilmington, MA, USA). Nude mice with hemizygote K14-hIL-8 transgene were screened for based on PCR (5'-GGCCCAAGGAGGACCAAG-3'/5'-AGGGATCTCCCTCAAAGGCTTC-3'), followed by digestion with BseDI and used for human colon cancer xenograft studies. CXCR2 KO mice in BALB/c background (C129S2(B6)-Cxcr2<sup>−/−</sup>Mwm/J) were purchased from the Jackson Laboratory (Bar Harbor, MN, USA).

In-vivo tumour growth study

One million CT26 cells in 100 µl phosphate-buffered saline (PBS) were injected into both the right and left flanks of wild-type (WT) control and CXCR2 KO mice (six mice per group). Two hundred and thousand CT26 cells in 100 µl PBS were injected into the right and left flanks of WT control and K14-hIL-8/nude mice (seven mice per group). One million of HCT116 cells in 100 µl PBS were injected into both flanks of WT control and K14-hIL-8/nude mice (five mice per group). Each tumour volume was measured by width (mm) and length (mm) every 2 days using a digital caliper (0.1 mm increments) until they were killed. Tumour volumes were calculated with the following formula: tumour volume = ((width<sup>2</sup> × length)/2).

Delayed-type hypersensitivity reactions

As previously described (Kunstfeld et al., 2004), 2% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazoline-5-one; Sigma, St Louis, MO, USA) solution in acetone/olive oil (4:1 vol/vol) was treated topically onto the shaved abdomens (50 µl) and onto each paw (5 µl) of 6- to 8-week-old mice, which did not display pre-existing inflammatory lesions. After 5 days, 20 µl of a 1% oxazolone solution was challenged on their right ears and the vehicle was treated on the left ears. The ear thickness (total nine mice per time point) was measured up to 16 days until swellings were all reduced.

Metastasis assay

One million HCT116LR cells were injected into the tail veins of the control WT and K14-hIL-8/nu mice (five mice per group). Mice were killed after 15 weeks for analyses. Non-invasive images of whole mice and then their organs (liver, lung, heart, spleen, kidney and subcutaneous lesions) were captured to detect luciferase and RFP signals by Molecular Imaging Center (University of Southern California), as previously described (Bhaumik and Gambhir, 2002; Wang and El-Deiry, 2003; Paroo et al., 2004). The total number of metastatic foci was counted and compared between the WT and K14-hIL-8/nu mice.

Immunohistochemistry and vascular analyses

At the end of study, tumour samples were harvested and processed for paraffin embedding. Anti-CD31 (AbCam, San Francisco, CA, USA; 1:1000) and hIL-8 (BioLegend, San Diego, CA, USA; 1:200) antibodies were used for IHC. Immunohistochemistry images were captured using AxioImager Z1 microscope (Zeiss, Oberkochen, Germany) and vascular analyses were performed using the NIH Imagej software (Bethesda, MD, USA).

RNA isolation and semi-quantitative RT–PCR

TRIzol Reagent (Sigma) was used to extract total RNAs from mouse skin. In all, 2 µg of tissue RNA was subjected to the standard RT–PCR procedures. The expression of mouse GAPDH was also detected as an internal control for normalisation. Primer sequences will be provided upon request.

Cell proliferation assay and ELISA

Cell proliferation was determined by increased cell number using the WST-1 assay kit (Fisher, Pittsburgh, PA, USA). In brief, cells were grown in a low serum media (DMEM plus 1% fetal bovine serum) and cell proliferation was measured every 2 days up to 16 days until the absorbance was stable.
serum) in the presence of DMSO (0.1%; Sigma), hIL-8 (10 ng ml^{-1}; R&D, Minneapolis, MN, USA), mouse Gro-z (10 ng ml^{-1}; Peprotech, Rocky Hill, NJ, USA) and/or SCH-527123 (synthesised by Dr Nicos Petasis). After 48 h, the cultures were treated with WST-1 for 3 h and then their absorbance was measured at 450 nm. For the ELISA, whole blood was collected from mouse tails and, after coagulation, centrifuged to obtain the serum. Serum levels of hIL-8 was determined using Human IL-8 ELISA development kit from BioLegend.

Statistical analyses

The outcome measures are expressed as the mean ± s.d. per experimental condition. Analysis of variance or a t-test was used to detect the differences in outcome measures across conditions for all in-vitro and in-vivo experiments. A mixed linear model with the autoregressive covariance structure overtime was used to compare tumour volume for the duration of the experiment by condition. The analyses were conducted using the SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC, USA). Two-sided P-values were reported for all tests at a significance level of 0.05.

RESULT

The IL-8 and CXCR2 signalling stimulate the proliferation of the CT26 and HCT116 colon cancer cells

To study the effect of the IL-8/CXCR2 signal on colon cancer cells, we chose the mouse colon carcinoma cell line CT26 and human CRC cell line HCT116. We first confirmed the expression of CXCR2 in the two cell lines by immunofluorescence (IF) staining (Figure 1A and C). The IF analyses without the anti-CXCR2 antibody failed to exhibit any signal, validating the specificity of the antibody for CXCR2 (Figure 1B and D). We then studied the proliferative responses of these two cancer cell lines to IL-8. Indeed, the addition of hIL-8 was able to stimulate cell proliferation of both the mouse and human colon cancer cells (Figure 1E and F). Next, a selective antagonist for CXCR1/2, SCH-527123 (Dwyer et al, 2006; Chapman et al, 2007), was used to confirm that the IL-8-activated cell proliferation was mediated through CXCR2, and further that SCH-527123, in the presence of IL-8, inhibits the proliferation of both cell lines (Figure 1G and H).

Generation and characterisation of a transgenic mouse line with a targeted expression of hIL-8 in the skin

In order to develop an animal model, in which IL-8 was steadily supplied by the tumour microenvironment, we generated a noble transgenic mouse (K14-hIL-8) that can express and secrete hIL-8 from the skin. The K14-hIL-8 mouse carries a transgene cassette that contains the hIL-8 open reading frame under the direction of the basal keratinocyte-specific keratin-14 promoter (Figure 2A). A combined analyses using enzyme-linked immunosorbent assay (ELISA) and PCR-based genotyping showed that >100 pg ml^{-1} of hIL-8 was detectable in the serum from the founder mice that carried the transgene cassette (Figure 2A). In addition, IHC analyses confirmed that hIL-8 was abundantly produced in the keratinocytes of the skin and diffused towards the subcutaneous area in the K14-hIL-8, but not in the WT nude mice (Figure 2B and C). In addition to these expression studies, we performed a functional assay to validate the activity of hIL-8 produced and secreted by the mouse skin cells. Since the IL-8/CXCR2 pathway has been known to play a major role in inflammation, we carried out the delayed-type hypersensitivity (DTH) assay, which allows us to assess the contribution of hIL-8 produced by mouse keratinocytes to cell-based inflammatory response. Ears of the WT and K14-hIL-8 transgenic mice were subjected to the Oxazolone-based DTH reaction and the thickness of the ears, an indicator of the severity of inflammatory responses, was measured every day thereafter. Indeed, while the vehicle-treated ears remained unchanged, Oxazolone-challenged ears developed severe swellings from day 1 in both genotypes of mice (Figure 2D). Importantly, however, the K14-hIL-8 transgenic mice exhibited a significant increase in ear swelling over the WT mice did during the first week. This finding indicates that the mouse keratinocyte-encoding hIL-8 in the transgenic mice was fully functional and could augment the DTH-induced inflammatory responses. Together, our novel K14-hIL-8 transgenic mice may serve as a useful animal model to create a tissue microenvironment with increased IL-8 expression.

Increased IL-8 levels in the tumour microenvironment promoted tumour growth and enhanced angiogenesis

We next set out to investigate the establishment of xenograft tumour formation and subsequent growth of mouse CT26 and human HCT116 colon cancer cells in the K14-hIL-8 transgenic mice. A potential limitation to this experiment was the possibility that when implanted into the K14-hIL-8 transgenic mice (B6D2 background), CT26 (BALB/c background) and HCT116 (human) cells may elicit immune responses, which will significantly influence tumour formation and growth as well as tumour-associated angiogenesis. To address this, we established immunodeficient K14-hIL-8 transgenic mouse line by crossing the K14-hIL-8 mice with athymic immunodeficient nude mice.

The immunodeficient K14-hIL-8 transgenic mouse model, termed K14-hIL-8/nu, was then used to investigate the impact of elevated IL-8 levels in tissue microenvironment on the formation and growth of mouse and human colon cancers. One million CT26 colon cancer cells were subcutaneously injected into either the K14-hIL-8/nu mice or non-transgenic WT nude mice. Importantly, the CT26 tumours in the K14-hIL-8/nu mice grew much faster when compared with those in the nude control mice (Figure 3A). Morphometric analyses of CD31-positive vessels revealed an increased vessel number in the peri-tumour area, but not within the tumour, of the K14-hIL-8/nu mice (Figure 3B). In addition, an increased vessel number in both peri-tumoural and intra-tumoural regions was detected in the K14-hIL-8/nu mice (Figure 3C). Similarly, one million HCT116 cells were subcutaneously injected into the flanks of both K14-hIL-8/nu and WT nude mice. Consistent with CT26 experiment, HCT116 cells in the K14-hIL-8/nu mice established tumours earlier and the resulting tumours grew much faster when compared with the WT nude mice (Figure 3D). In addition, vascular analyses revealed a statistically significant increase in CD31-positive vessel number only in the peri-tumoural area, but not in the intra-tumoural region (Figure 3E). We did not find any statistically significant changes in the microvascular density (Figure 3F). Importantly, no changes in the vessel number in the intra-tumoural area between these two mouse groups was observed, in noteworthy contrast to the significant vessel number increase by tumour cell-derived IL-8, as our recent study showed (Ning et al, 2011). This suggests that the IL-8 supplied by the tumour microenvironment activates angiogenesis predominantly in the peri-tumoural area rather than the intra-tumoural region.

Enhanced metastatic potential of human colon cancer cells in animal models with increased serum levels of IL-8

Because our previous clinical data showed that patients with metastatic CRC exhibit >10 times higher serum levels of IL-8 when compared with normal individuals (Ning et al, 2011), we next studied the impact of increased serum IL-8 levels on the metastatic potential of HCT116 cells. In particular, we aimed to assess the ability of HCT116 colon cancer cells to extravasate into distant organs and then to establish metastatic foci and secondary metastases. To this end, we used the K14-hIL-8/nu mice for the experiments. A mixed linear model with the autoregressive covariance structure over time was used to compare tumour volume for the duration of the experiment by condition. The analyses were conducted using the SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC, USA). Two-sided P-values were reported for all tests at a significance level of 0.05.

Effect of IL-8 on microenvironment on colon cancer growth

YS Lee et al

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tumours. We engineered HCT116 cells to stably express both firefly luciferase and RFP by lentivirus transduction (Figure 4A). These labelled HCT116 cells (HCT116LR) were injected into the tail veins of K14-hIL-8/nu or WT nude mice. After 7 weeks, both the whole mice and individual organs were subjected to bioluminescent/fluorescent imaging analyses (Figure 4B–D). Micrometastatic foci and secondary tumours were counted in two major metastatic lesions sites (the lung and liver) and other organs of K14-hIL-8/nu or WT nude mice. It was indeed found that the HCT116LR colon cancer cells had an increased metastatic potential in the K14-hIL-8/nu mice when compared with the WT nude mice (total incidence: 4.2 vs 0.75, \( P < 0.05 \)) (Figure 4E).

Both colon cancer growth and angiogenesis was inhibited by CXCR2 deletion in the tumour microenvironment

Interleukin-8 is known to bind to and activate the high affinity receptor, CXCR2. Previous studies showed delayed wound healing and reduced lung cancer growth in CXCR2 KO mice (Devalaraja et al, 2000; Keane et al, 2004). More recently, CXCR2 inhibition was found to disrupt tumour-stromal interactions and also improve survival in a pancreatic ductal adenocarcinoma mouse model (Ijichi et al, 2011). We have reported that a CXCR2 polymorphism may be served as a molecular marker for PFS in ovarian cancer patients (Schultheis et al, 2008). Moreover, we also reported another CXCR2 polymorphism that can predict clinical
outcome and tumour response in metastatic CRC patients treated with bevacizumab and oxaliplatin-based chemotherapy (Gerger et al., 2011). Another germline polymorphism in CXCR2 was identified to play a pivotal role in the epidermal growth factor receptor pathway (Zhang et al., 2005).

Thus, we next determined the role of CXCR2 in the tumour microenvironment in colon cancer cell growth using syngeneic CXCR2 KO mice. Equal number of CT26 cells (BALB/c background) were subcutaneously injected into the left and right flanks of the WT or CXCR2 KO mice, both in the BALB/c background and
tumour growth was measured for 15 days (Figure 5A). Notably, tumours in both groups of mice grew in a comparable rate until they reached a volume of \( \approx 100 \) mm\(^3\). Thereafter, tumours in the WT mice grew faster than those in CXCR2 KO mice (Figure 5A). Vascular analyses revealed that tumours in the CXCR2 KO mice showed a reduced average vessel number/area in the peri-tumoural region and a reduced average vessel size in the intra-tumoural area (Figure 5B, C and F). Moreover, we found increased necrotic area in tumours in the CXCR2 KO mice, compared with the WT mice (Figure 5D and E). A previous study has shown that depletion of

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**Figure 4**  Enhanced metastatic potential of human colon cancer cells in K14-hIL-8/nu mice (A) Bright field, red fluorescent and combined images of cultured HCT116 cells (× 100) (B) RFP signals from both WT nude and K14-hIL-8/nu mouse. Both mice were skinned to better detect the RFP signal. (C) Liver, lung, heart, kidney, spleen and subcutaneous lesions were harvested from K14-hIL-8/nu mice and their bioluminescent signals from firefly luciferase and fluorescent signals from RFP were simultaneously captured. (D) Bright field and red fluorescent images showing lung metastases in the WT nude and K14-hIL-8/nu mice. (E) Average number of metastatic lesions in the lung, liver and other organs (skin, heart, spleen and kidney) in the WT nude (\( n = 5 \)) and K14-hIL-8/nu (\( n = 6 \)) mice. Graph represents the average number of total metastases in the WT nude vs K14-hIL-8/nu mice. \( *P < 0.05 \). The colour reproduction of this figure is available at the British Journal of Cancer online.
CXCR1 led to downregulation of CXCR2 at the transcription level without a reciprocal regulation of CXCR1 by CXCR2 (Shamaladevi et al, 2009). We examined the expression level of CXCR1 mRNA in the CXCR2 KO mice and confirmed the absence of regulation of CXCR1 by CXCR2 at least at the transcriptional level (Figure 5G). Taken together, our studies demonstrate that the deletion of CXCR2 in the tumour microenvironment profoundly affects colon cancer growth and accompanying development of angiogenesis, and further increases intra-tumoural necrosis, providing evidence of the essential roles of CXCR2 in colon cancer development and progression.

**DISCUSSION**

Colorectal cancer is the leading cause of death from gastrointestinal malignancy and second most common cause of cancer-related death in the United States. Interleukin-8 and its receptor, CXCR2, are significantly upregulated in various stages of CRC and their respective tissue microenvironments. From patient specimens acquired through translational and clinical trials, we have reported that genetic variations in IL-8 and CXCR2 genes profoundly affects CRC progression, drug sensitivity and tumour recurrence (Gordon et al, 2006; Lurje et al, 2008; Schultheis et al, 2008; Zhang et al, 2009). This capacity to determine clinical outcomes highlights the evolving role of IL-8/CXCR2 as the molecular predictors of response, in particular with regards to patient responsiveness towards anti-VEGF and oxaliplatin-based chemotherapy (Zhang et al, 2003, 2005, 2006; Vallbohmer et al, 2005; Gordon et al, 2006; Lurje et al, 2008). We have also identified that IL-8 may serve as a key factor capable of promoting CRC proliferation and progression by stimulating tumour cell proliferation, invasion, migration and angiogenesis (Ning et al, 2011).

Our data using novel animal models demonstrate for the first time that the expression of IL-8/CXCR2 in the tumour microenvironment plays a critical role in colon cancer growth, progression and metastases. We and others previously had shown that polymorphisms in the IL-8/CXCR2 genes that elevates serum levels of IL-8 were associated with colon cancer progression, recurrence and drug resistance (Hull et al, 2000; McCarron et al, 2002; Lee et al, 2005; Obuyauchi et al, 2005; Taguchi et al, 2005; Gordon et al, 2006; Snoussi et al, 2006; Lurje et al, 2008; Schultheis et al, 2008; Zhang et al, 2009; Ning et al, 2011). Importantly, we have recently reported that tumour cell-derived IL-8 promotes colon cancer growth in vitro and in vivo and that advanced colon cancer patients exhibit 10 times higher serum levels of IL-8 when compared with normal individuals (Ning et al, 2011). However, these studies suggest that the regulation of IL-8 within the tumour and microenvironment play a critical role, but the impact of tissue microenvironment-derived IL-8 and CXCR2 to date has been difficult to evaluate.

In this study, we investigated the effect of increased expression of IL-8 in the tumour microenvironment on colon cancer growth by generating a novel immunodeficient, transgenic mouse
(K14-hIL-8/nu) that expresses hIL-8 in the skin. Human and mouse colon cancer cells were seeded in the IL-8-rich tissue environment of the K14-hIL-8/nu skin and their tumour formation and growth were studied. Both human and mouse colon cancer cells were found to form tumours with increased volume in the IL-8-rich tissue environment when compared with the normal tissue environment. Interestingly, IL-8 supplied from the tumour microenvironment stimulated the peri-tumoural, but not intra-tumoural, angiogenesis, unlike tumour cell-derived IL-8, which activated angiogenesis in the entire tumour area (Ning et al., 2011). Moreover, the increased serum expression of IL-8 was also found to enhance cancer cell extravasation into the lung and liver tissue and growth of secondary tumours therein. This finding is particularly important in the context of our previous finding of an increased serum level of IL-8 in patients with metastatic CRC (Ning et al., 2011), which suggests that the systemic increase of IL-8 may be more important for the prognosis than the local, tumour cell-derived IL-8 levels. Moreover, our study with the CXCR2 KO mouse model further supports the profound role of the microenvironment-derived IL-8/CXCR2 in colon cancer growth. In addition to IL-8, CXCR2 can be activated by other cytokines such as Gro-α/CXCL1, which has been also adversely associated with colon cancer development and progression (Cuenca et al., 1992; Baier et al., 2005; Wen et al., 2006; Ogata et al., 2010). Therefore, our current studies verify utilising two mouse models the previously speculated, but not yet proven role that the microenvironment-mediated IL-8 and CXCR2 play in colon cancer growth and metastasis.

Importantly, activated IL-8/CXCR2 in colon cancer confers a resistance to the anti-VEGF therapy: IL-8 maintains its proangiogenic activity even in HIF1α-deficient colon cancer cells, where VEGF expression was compromised and IL-8 expression was significantly induced by production of hydrogen peroxide and subsequent activation of NF-κB (Mizukami et al., 2005). This finding indicates that IL-8 plays a key role in tumour angiogenesis independent of VEGF, and operates even when VEGF is limited or inactivated, suggesting a mechanism underlying colon cancer resistance against anti-VEGF therapy (Mizukami et al., 2005). Moreover, the KRAS oncogene, which is frequently mutated in colon cancer, enhances the hypoxic induction of IL-8 and a neutralising antibody against IL-8 substantially inhibited angiogenesis and tumour growth (Mizukami et al., 2005). Taken together, the IL-8/CXCR2 signal pathway has been demonstrated to be a promising target for colon cancer since inhibition of this pathway may not only lead to antitumour properties, but may also chemosensitise the tumour toward the current chemotherapy.

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Conflict of interest

The authors declare no conflict of interest.

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