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Original contribution

Expression of angiostatic platelet factor-4var/CXCL4L1 counterbalances angiogenic impulses of vascular endothelial growth factor, interleukin-8/CXCL8, and stromal cell-derived factor 1/CXCL12 in esophageal and colorectal cancer☆

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Summary Chemokines influence tumor progression through regulation of leukocyte chemotaxis, angiogenesis, and metastasis. In this study, the regulated expression of angiogenic (stromal cell-derived factor [SDF]-1/CXCL12 and interleukin [IL]-8/CXCL8) and angiostatic (platelet factor [PF]-4var/CXCL4L1 and inducible protein [IP-10]/CXCL10) chemokines was examined in human colorectal and esophageal cancer. In HCT 116 and HCT-8 colorectal adenocarcinoma cells, the production of IL-8 immunoreactivity was up-regulated by IL-1β, tumor necrosis factor (TNF)-α, the toll-like receptor (TLR) ligands double-stranded RNA and peptidoglycan and phorbol ester. Increased PF-4 and synergistic IL-8 and IP-10 induction in carcinoma cells after stimulation with IL-1β plus TNF-α or interferon-γ was demonstrated by enzyme-linked immunosorbent assay, quantitative reverse transcriptase polymerase chain reaction, or immunocytochemistry. In addition, IL-8 from HT-29 colorectal adenocarcinoma cells was molecularly identified as intact chemokine, as well as NH_2-terminally truncated, more active IL-8(6-77). The presence of PF-4var, SDF-1, and vascular endothelial growth factor (VEGF) was evidenced by immunohistochemistry in surgical samples from 51 patients operated on for colon adenocarcinoma (AC), esophageal AC, or esophageal squamous cell carcinoma (SCC). PF-4var was strongly detected in colorectal cancer, whereas its expression in esophageal cancer was rather weak. Staining for SDF-1 was almost negative in esophageal SCC, whereas a more intense and frequent staining was observed in AC of the esophagus and colon. Staining for VEGF was moderately to strongly positive in all 3 types of cancer, although less prominent in esophageal AC. The heterogenous expression of angiogenic (IL-8, SDF-1) as well as angiostatic (IP-10, PF-4var) chemokines not

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only within the tumor and between the different cases but also between the different tumor cell types may indicate a distinct role of the various chemokines in the complex process of tumor development.

1. Introduction

Chemotactic cytokines, shortly chemokines, are small proteins consisting of 70 to 125 amino acids that can be subdivided in 2 major subclasses, CXC and CC chemokines, depending on whether the first 2 cysteine residues are separated by an amino acid (CXC) or are adjacent (CC) [1,2]. Most if not all chemokines exert their functions through the binding to G protein-coupled receptors designated CXCR or CCR [1,3]. Chemokines exhibit multiple functions such as chemotaxis, regulation of hematopoiesis, angiogenesis, and tumorigenesis [2]. Cancer is a multistep process in which oncogenes are activated and tumor suppressor genes are inactivated. These changes give the cells new properties such as hyperactive growth and immortality through protection against programmed cell death [4,5]. Altered gene transcription can switch on the production of chemokines that modulate tumor behavior by 3 important mechanisms: regulation of angiogenesis, activation of a tumor-specific immune response, and direct stimulation of tumor proliferation in an autocrine fashion [6].

The regulated production of abundant chemokines such as angiogenic interleukin-8 (IL-8)/CXCL8 and angiostatic interferon (IFN)-γ inducible protein-10 (IP-10)/CXCL10 by different human colorectal adenocarcinoma cell lines is already well-known [7-10]. Angiogenic stromal-cell derived factor-1 (SDF-1)/CXCL12 has up to now only been found in the more differentiated colorectal adenocarcinoma cell line Caco-2 [7,11]. No data are presently available on the expression of less abundant or more recently identified chemokines such as the angiostatic platelet factor-4 variant (PF-4var)/CXCL4L1 in human epithelial tumors.

This study focuses on the expression of several angiogenic and angiostatic chemokines in human colorectal and esophageal cancer. We tested different colorectal adenocarcinoma (AC) cell lines (COLO 320 and HCT 116) and the ileocecal AC cell line HCT-8 for the expression of PF-4var, SDF-1, IL-8, and IP-10 using different techniques such as enzyme-linked immunosorbent assay (ELISA), quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and immunocytochemical staining after induction with cytokines, cytokine inducers, and toll-like receptor ligands. It was further verified which molecular forms of IL-8 were produced by the human colorectal AC cell line HT-29, previously described to produce IL-8 [12-14]. Finally, we studied the expression of PF-4var, SDF-1, and vascular endothelial growth factor (VEGF) in epithelial tumors by immunohistochemical staining on tumor biopsy specimens derived from 51 patients with AC of the colon, AC of the esophagus, or squamous cell carcinoma (SCC) of the esophagus.

2. Materials and methods

2.1. Induction experiments and ELISAs

Cultures of human AC cell lines (COLO 320, HCT 116, HCT-8) were stimulated with different concentrations of cytokines, cytokine inducers, combinations of these inducers, or were left untreated [12,15]. After 72 hours, the conditioned media were harvested in which concentrations of human PF-4 plus PF-4var, IL-8, IP-10, and SDF-1 were determined using sandwich ELISAs, as described previously [16].

2.2. Quantitative reverse transcriptase polymerase chain reaction

HCT-8 cells were induced for 16 hours and isolation of messenger RNA (mRNA) and qRT-PCR was performed as described previously [17]. Thrombin stimulated blood platelets were used as positive control. The mRNA levels of PF-4 (Hs00427220_g1), PF-4var (Hs00601249_g1), and IL-8 (Hs00174103_m1) were determined with commercial TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA).

2.3. Purification, ion-trap mass spectrometry, and NH_2-terminal sequence analysis

To purify IL-8, conditioned medium of HT-29 cells, induced with 0.5 ng/mL of IL-1β was purified as described by Struyf et al [15].

2.4. Immunocytochemistry and immunohistochemistry

Immunocytochemical staining of chemokines in AC cell lines was performed as described by Vandercappellen et al [16].

For histology and immunohistochemistry, archived material was used from the Department of Pathology. Patients who underwent resection for AC of the colon included 10 men and 10 women (mean age, 60 years). The patients who had AC of the esophagus were 14 men and 4 women (mean age, 65.1 years). Patients who were operated on for SCC of the esophagus included 10 men and 3 women (mean age, 60.2 years). Both sections from normal and neoplastic tissue were used for the study. In patients with AC of the esophagus, we also examined the metaplastic intestinal-type columnar mucosa, which is considered to be a precursor lesion. The tumors were classified and staged according to the World Health Organization histologic classification and the TNM