Matrix metalloproteinases in inflammatory bowel disease : expression, regulation and clinical relevance

Meijer, M.J.W.

Chapter 1

Introduction

Inflammatory Bowel Disease - a major health problem

Inflammatory bowel disease (IBD), i.e., Crohn's disease (CD) and ulcerative colitis (UC), are characterized by an idiopathic, chronic and recurrent inflammation of the gastrointestinal tract. In CD, inflammation is segmental and transmural, often localized in (but not confined to) the ileocaecal area, while UC is limited to the mucosal lining of the colon, often starting in the rectum and extending in proximal direction through the years. In both CD and UC, inflammation may result in severe tissue damage, i.e., discontinuation of the epithelial border, ulcera, fissures, loss of circular folding, cobblestone appearance, fibrosis, stenosis and in CD also the formation of entero-entero, entero-viscero or entero-cutaneous fistulae. Abdominal pain, increased defecation frequency, bloody diarrhea, nausea, significant body weight loss and anemia related fatigue all contribute to the general malaise IBD patients often experience. Disease course may be complicated by osteoporosis, arthritis, ankylosing spondylitis, iritis, uveitis, erythema nodosom and primary sclerosing cholangitis (UC). Patients are at increased risk for developing colorectal carcinoma[1,2] and recently, IBD was associated with a higher incidence of adverse pregnancy outcomes.[3] First line of treatment consists of 5-ASA containing mesalasine/sulphasalazine or corticosteroid budesonide/prednisolone tablets, enema or suppository. In corticosteroid refractory patients disease modifying drugs azathioprine, cyclosporin, methotrexate or mycophenolate mofetil are administered. The therapeutic arsenal for CD patients has been expanded recently with biological agents specifically targeting TNF-α in the immune cascade. Chimeric (75% human/25% murine) infliximab and humanised adalumimab anti TNF-α antibodies have proven to be effective in the clinical setting for steroid dependent/refractory CD patients.[4-6] Disadvantages include the large placebo effect requiring further optimalization, host antibody response to infliximab and high medical costs.[7,8] Biological agents against IL-12, adhesion molecules and IL-6 receptors are promising new candidates but are not expected to enter the market soon.[9,10] Despite advances in treatment protocols, the natural history of the disease appears unmodified.[11,12] Cumulative 10 year surgical resection probability rates vary from 25-60 % in CD and 25% in UC patients and these percentages have remained similar for 4 decades although surgical resection rates within the first year of

diagnosis might be decreasing.[13-16] The socioeconomic burden remains high: in the United States total costs of IBD related healthcare are approximately 1 billion US dollar per year.[17] When work-productivity losses due to chronic disability are taken into account, total costs of IBD in the USA were estimated $ 5 billion in 2000.[18] Therefore, much effort is spent in research aimed at identifying the epidemiology, etiology and pathogenic mechanisms underlying IBD.

Inflammatory Bowel Disease - epidemiology & etiology

Annual incidence rates of CD in white residents of Western countries vary between 4-9 per 100,000, whereas the incidence of UC appears somewhat higher (9-14 per 100,000), with concordant prevalence rates of 130-175 (CD) and 240-275 (UC) per 100,000 persons.[16,19-22] Studies from Canada and New Zealand, however, reported higher incidence figures for CD compared to UC (14.6 versus 14.3 and 16.5 versus 7.6 per 100,000, respectively).[23,24] In both CD and UC, distribution of age at onset of disease is skewed, with an incidence peak between 20-30 years. Incidence and prevalence rates have risen after the Second World War, reaching the current plateau in the 1970s, affecting more people in urban versus rural areas.[25,26] People in white collar occupations appear more at risk compared to other groups in the population.[27] A north-south gradient has been postulated,[28] but in industrializing countries, incidence and prevalence of IBD are also increasing,[29] as are disease rates among Asian immigrants in Western countries.[30] Although disease concordance rates in monozygotic twins are high (CD: 60, UC: 20 %), pointing to a genetic influence, these sub maximum figures also suggest the involvement of one or several environmental factor(s) in the etiology of IBD.[31] Smoking was established as such a factor, worsening the prognosis in CD[32] and instead protective in UC.[33] Other studies have suggested an association of CD and/or UC with infectious agents such as *Mycobacterium avium sp. Paratuberculosis*,[34] invasive *Escherichia coli* strains[35,36] and measles virus infection or vaccination.[37-39] In case of *Helicobacter pylori*, a protective effect was suggested.[40] Married couples are at greater risk of sequentially contracting the disease after cohabitation.[41] Persons born in the winter months were more at risk for IBD[42] and onset of symptoms was also especially observed during this period, thus arguing for the

rationale of an infectious agent involved in the etiology of IBD,[43] although this remains to be confirmed.[44] Other studies have focused on an association with dietary food intake, such as breastfeeding, fast food, coca cola beverages and chocolate consumption.[45-47] Use of oral contraceptives,[48,49] menstrual cycle[50] and psychological distress[51] have also been implicated, again with inconclusive or even contradictory results.[52] Probably, the rise in incidence of IBD after the Second World War in Western and industrializing countries might be attributed to the introduction of better sanitary conditions, thus improving survival of susceptible individuals and/or shifting the development of the immune system towards hypersensitivity.[53-55] It should be noted that incidence and prevalence of asthma and diabetes type I have also increased, pointing to a more general promoting effect of western lifestyle on auto-immune disease.[56]

Several studies indicate genetic susceptibility in the etiology of IBD as well. For instance, IBD concordance rates are higher in monozygotic versus dizygotic twins,[31] IBD affecting multiple family members is frequently seen,[57] and incidence rate of IBD appears to be associated with ethnicity and religion group, as demonstrated by the increased prevalence in white Caucasian subjects and Ashkenazi Jews compared to blacks, Asians and Hispanics.[58-60] Early genome-wide association studies have identified (potential) IBD loci on chromosomes 1, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19, 22 and X.[61-66] Subsequent fine-mapping has revealed the involvement of the nucleotide-binding oligomerisation protein 2 (NOD2)/ caspase activation and recruitment domain 15 (CARD15) gene on chromosome 16q12 in CD susceptibility,[67,68] which was confirmed in other studies.[69,70] Interestingly, single nucleotide polymorphisms (SNPs) within this gene were also found to be associated with asthma,[71] Blau syndrome,[72] increased mortality following sepsis[73] and allogeneic stem cell transplantation.[74,75] This landmark success and the increasing availability of high density SNP arrays led to a surge in genome wide research and to the identification of other loci strongly implicated in the pathogenesis of CD and/or UC, including the gene encoding a subunit for the IL-23 receptor (IL-23R) on chromosome 1p31,[76,77] genes involved in autophagy/breakdown of intracellular pathogens (autophagy related 16-like 1 gene (ATG16L1) on 2q37 and immunity related p47 guanosine triphosphatase murine

orholog (IRGM) on 5q33,[78-82] a gene desert on 5p13 regulating expression of the prostaglandin receptor EP4 (PTGER4) (83), the nel-like 1 precursor (NELL1) gene on 11p15[84] and just recently 3p21.31, NKX2-3, CCNY (CD and UC) and PTPN2, HERC2 and STAT3 (UC).[85] Importantly, these studies were conducted in Caucasian populations and not replicated in Asian IBD patients, reinforcing the pivotal role of ethnicity in this matter.[86,87] Other genes potentially involved in the susceptibility and/or phenotype of CD and/or UC are derived from candidate gene approach and include those encoding interleukin-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-10, IL-11, tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NFκB), Toll like receptors (TLR), discs large homolog 5 (DLG-5), mucins, organic cation transporter-1 and -2 (OCTN-1, -2), mannan binding lectin (MBL), multidrug resistance 1 protein (MDR) and pregnane X receptor (PXR).[88-102] Occasionally, conflicting data were generated, probably due to the relatively small sample size often used, especially in the early studies.[103-106] Also, because of the nature of this approach, significant results might indicate the association of an adjacent predisposing gene in linkage disequilibrium with the examined gene, complicating this matter. However, it appears that, in cooperation with selected environmental stimuli, different sets of predisposing genes might give rise to essentially the same clinical disease manifestation, collectively called IBD. This is corroborated by the large number of mouse models, where targeting (i.e., IL-10) or overexpressing (i.e., TNF-α) of different genes leads to similar IBD like disease.[107-112]

Inflammatory Bowel Disease - pathogenic mechanisms

A widely accepted hypothesis states that IBD is an exaggerated immune response towards commensal bacteria in genetically susceptible individuals.[113] The recent rise in incidence of both forms of IBD might be attributed to increased exposure to so-called psychrotrophic bacteria by introduction of refrigerated food (cold chain hypothesis).[114] The concept of commensal bacteria playing a pathogenic role is corroborated by the increased frequency of anti-*Escherichia coli* outer membrane porin C, anti-flagellin and autoreactive mycobacterial HSP65 antibodies in CD and/or UC.[115-117] Flagellin specific T cells were able to cause fulminant colitis in an adoptive mouse transfer model.[118] Moreover, murine models for IBD do not

develop enteritis given a pathogen-free environment, although this was just recently challenged by the development of chronic ileitis in germ/free SAMP1/YitFc mice, suggesting that bacteria exacerbate disease but are not required for induction.[119] In CD patients, expression of anti-bacterial α-defensins HD5 and HD6 by ileal Paneth cells is reduced, especially in NOD-2 mutation carriers.[120] The levels of mucus forming proteins mucin3, 4, 5B and of sealing tight junction proteins claudin 5 and -8 are downregulated, whereas expression of pore-forming claudin 2 and rate of epithelial cell apoptosis are increased, resulting in impaired mucosal barrier function.[121,122] After epithelial injury, in CD patients an abnormally low neutrophil accumulation was observed compared to healthy controls, suggesting an impaired innate immune response.[123] The impaired mucosal barrier and/or the impaired innate immunity might result in overexposure to commensal bacteria, initiating and/or propagating the uncontrolled adaptive immune response seen in IBD. Importantly, IBD T-lymphocytes and neutrophils demonstrate increased resistance to apoptosis, thus sustaining the immune response.[124-127] Also, T cell regulatory (Treg) function might be insufficient to dampen the inflammatory reaction.[128] Both diseases are characterized by upregulation of pro-inflammatory cytokines (i.e., IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-15, -16, -17, -32, TNF-α, IFN-γ), chemokines (MIP1α, MIP1β, MIP3α, MCP1, MCP2, RANTES), neuropeptide substance P, growth factors (bFGF, VEGF, KGF), eicosanoid PGE2, corresponding receptors (TNF-R2, neurokinin-1/substance P receptor) and endothelial/leucocyte adhesion molecules (ICAM-1, selectins, LFA-1, α4β7 integrin/MAdCAM) in the (inflamed) intestinal mucosa, not compensated for by anti-inflammatory cytokines, soluble receptors and/or receptor antagonists (i.e., IL-10, TGF-β1, sTNFR, sIL1-RII, sgp130, IL-1RA).[129-156] However, some important differences are observed in the cytokine profile between CD and UC (upregulation of IL-12, IL-23 versus IL-13, respectively), reflecting the Th1 versus Th2 nature of the corresponding disease.[157-160] The cytokine expression in IBD is different in chronic versus early lesions, thus complicating this issue.[161,162] The upregulation of cytokines, chemokines, neuropeptides, growth factors, receptors and adhesion molecules chronically activates resident mesenchymal, epithelial and immune cells and continuously attracts new leucocytes from the peripheral circulation. In the

battle against their unknown targets, these cells may damage the intestinal tissue in various ways. T cell activated neutrophils and macrophages release massive amounts of harmful reactive oxygen metabolites by NADPH-dependent oxidative burst, targeting membrane lipids, protein and DNA, thus disrupting cellular structure at the molecular level and promoting malignancy.[163-166] An imbalanced anti-oxidant response in IBD patients may exacerbate disease.[167-169] Cytotoxic CD8+ T cells release pore forming perforin, proteolytic granzymes and/or express Fas ligand, triggering apoptosis in epithelial cells and disrupting mucosal barrier function.[170-172] Cytotoxic perforin releasing CD4+ T cells were demonstrated in CD[173,174] and activated complement in conjunction with IgG1 auto antibodies against tropomyosin isoform 5 may target epithelial cells in UC.[175,176] Increased expression of tissue remodeling neutrophil elastase by neutrophils and of chymase and tryptase by mast cells is associated with IBD.[177-179] Synthetic elastase and tryptase inhibitors were found beneficial in experimental colitis.[180,181] Concurrent attenuated induction of serine anti-proteases might exacerbate disease.[182] All activated cells also release specific members of the tissue remodeling Matrix Metalloproteinases (MMP), which are described below.

Matrix metalloproteinases - classification

Based on the catalytic group at the active center, five classes of proteases are recognized, i.e., serine-, threonine-, cysteine-, aspartic- and metallo-proteases, divided into clans and families based on protein folding and sequence similarity, see also http://merops.sanger.ac.uk/.[183] Clan MA of the metalloproteases is divided into subclans MA (M) and MA(E). Proteases designated to subclan M all contain a conserved methionine residue to the carboxy side of the active center, thus forming a characteristic loop or “Met turn” in the protein secondary structure, providing the base of the active cleft and are therefore called metzincins.[184] The metzincins are currently categorized into 12 families, each split into a variable number of subfamilies. Subfamily M12B contains the ADAMs (A Disintegrin And Metalloproteinase) and ADAMTSs (A Disintegrin-like And Metalloproteinase with Thrombospondin type 1 motifS).[185,186] It includes TACE (TNF-α Converting Enzyme, ADAM-17), which is important in releasing membrane-bound TNF-α and

TNF-R from the cell membrane[187] and ADAMTS-4 and -5, which cleave aggrecan in cartilage and might contribute to the structural damage seen in human arthritis.[188-190] Subfamily M10A, also called the matrixins, contains the matrix metalloproteinases (MMP). The human genome currently comprises 23 different MMPs, according to substrate specificity and protein structure subdivided into the collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT) MMPs, and a rest group (Table 1, page 24-26).

Matrix metalloproteinases - structure

All MMPs consist of a pre-, pro- and catalytic domain and, apart from MMP-7, -23 and -26, also contain a hinge region of varying length connecting a hemopexin domain (Table 1).[191,192] All MMPs except MMP-23 have a conserved sequence around cysteine in the propeptide (PR**C**GXPD), which is also found in the ADAMs and ADAMTSs. The cystein in this motif maintains the latency of the MMP. Limited proteolysis of the pro-peptide, treatment with chaotropic agents or organomercurials (APMA: *p*-amino-phenyl-mercuric acetate is widely used in *in vitro* experiments) disrupts the cystein-zinc bond and actives the enzyme.[193] A furin recognition motif (RX(R/K)R) is present on the carboxy terminus of the cysteine switch motif in several MMPs, allowing intracellular activation by furin-like pro-protein convertases in the Golgi apparatus. Recently, furin mediated inactivation of MMP-2 was observed and it appears other MMPs previously not recognized as furin substrates may also be targets.[194] The catalytic domain contains a conserved active site sequence: **H**EXX**H**XXGXX**H** with three histidine residues depicted in bold binding the zinc atom. The conserved sequence is shared with all other members of the metzincin group. MMP molecules contain additional non-catalytic zinc and calcium ions, which are involved in stabilizing the tertiary structure of the enzyme.[195,196] In the catalytic domain of MMP-2 and -9 three fibronectin type II repeats are inserted which bind gelatin and collagen thus facilitating the breakdown of these substrates. The flexible O-glycosylated proline-rich linker of MMP-9 is exceptionally large and facilitates binding of the enzyme to TIMP and cargo transporters.[197] The hemopexin domain co-determines substrate specificity and affinity. It binds Tissue Inhibitor of Metalloproteinases (TIMP, natural inhibitors of

MMPs) and docking molecules on cell surface membranes, for instance integrins and CD44. The hemopexin domain is also involved in MMP di-/oligomerization and CD97 mediated MMP uptake and internal degradation.[198] The MT-MMPs are connected to the cell by a short hydrophobic transmembrane segment or alternatively are glycosylphoshatidylinositol (GPI) anchored, focusing the cell's proteolytic capacity. However, cleavage at the stem region by other MMPs or ADAMs may release an active ectodomain.[199,200] The cysteine array and immunoglobulin-like domain in MMP-23 are of unknown function. The structural organization of the MMPs is also observed in the ADAMs and ADAMTSs, but these enzymes lack the hemopexin domain and possess other C-terminal extensions instead.[186]

Matrix metalloproteinases - expression

The expression of MMPs is tightly controlled at the transcriptional, translational and/or secretory level. Pro-inflammatory cytokines, chemokines, growth factors and oxidants including IL-1α, IL-1β, IL-6, IL-12, TNF-α, bFGF, MCP-1 and manganese superoxide dismutase (Mn-SOD) generated H_2O_2 upregulate the expression of several members of the MMP family including but not limited to MMP-1, -2, -3, -9, -12 and/or -13 in a variety of cells encompassing fibroblasts, enterocytes, T cells, chondrocytes, osteoblasts, endothelium and/or macrophages, sometimes in a synergistic manner.[201-211] Anti-inflammatory (TGF-β), pleiotropic (IFN-γ) cytokines and steroid sex hormones (estradiol) may downregulate MMP, whereas other members of the MMP family (i.e., MMP-2) display a rather constitutive expression rate in distinct cell types.[212-215] Importantly, results for a given combination of cytokine and MMP are not only dependent on cell type but also at what time point in their development and under what (experimental) conditions these cells are studied. Expression is also regulated by cell contact with the surrounding matrix, neighboring cells and pathogens. For instance: collagen I is able to induce MMP-1 in migrating keratinocytes thus promoting its own degradation and ligation of the fibronectin receptor α4/5β1, CD40 and TLR-2 may induce MMP-9.[216-220] The extrinsic signals are relayed to the MMP promoter by one or more intracellular signaling pathways, including NFκβ, SMAD, STAT, MAPK kinase pathways and

are integrated at cis-acting elements in the promoter, resulting in altered mRNA transcription rate.[221] Promoter activity is also dependent on DNA and histone methylation, acetylation and/or phosphorylation status.[222,223] Also, single nucleotide polymorphisms may result in the loss or gain of suppressor/enhancer DNA elements, affecting mRNA transcription. For instance, the replacement of cytosine with thymidine at -1306 in the MMP-2 promoter disrupts an Sp1 binding site, resulting in significantly decreased promoter activity.[224] A single guanine insertion at -1607 of the MMP-1 promoter creates an Ets binding site, elevating the transcriptional level of MMP-1.[225] The 3'UTR may contain ARE elements binding Hu and KH type splicing regulatory protein (KSFP) proteins increasing or decreasing mRNA stability, respectively, a mechanism shared with TNF-α and other pro-inflammatory cytokines.[226] The 3'UTR of MMP-9 mRNA is involved in binding cytoplasmic nucleolin, promoting transport to polyribosomes and enhancing protein translation efficiency.[227] Neutrophils and eosinophils store MMP-8, -9 and/or MT6-MMP in secretory granules which are released upon stimulation with pro-inflammatory cytokines such as IL-8 and TNF-α.[228-230] MT-1 MMP is stored in trans-golgi network/endosomes and may be expressed on the cell surface within minutes following Concanavalin A treatment of HT1080 cells.[231] Vesicular trafficking from the Golgi apparatus to the plasma membrane is dependent on actin and tubulin polymerization and can be suppressed by exposure to hypoxia, with concomitant drastic reduction of MMP secretion in monocytes.[232]

Matrix metalloproteinases - activation, inhibition and degradation

Except for furin-like pro-protein convertase-activated MT-MMPs and MMP-11, all other MMPs are secreted as inactive zymogens. Limited proteolysis by plasmin, thrombin, trypsin and other proteinases removes part of the propeptide region, inducing a conformational change in the MMP molecule disrupting the bond between the protective cysteine and catalytic zinc residue. Autocatalysis subsequently removes the entire propeptide region after which the enzyme becomes fully active.[233] The cysteine switch dogma is challenged by observations

of mutant MMP-3 with the cysteine replaced by serine or histidine. These molecules retained latency and could be activated with APMA, results not consistent with cysteine as primary regulator of MMP latency.[234] The final MMP activity is dependent on the activating proteinase, i.e., MMP-3-activated MMP-1 displays a higher conversion rate of collagen substrate compared to plasmin-activated MMP-1.[235] Contact with substrate or even non-functional protease may induce conformational change and activation of MMP without loss of the pro-peptide.[236,237] Oxidative activation and inactivation may play an important role during inflammation.[238] MMP-2 can be activated as described above, but the activation pathway in a complex with MT-MMPs and TIMP-2 is believed to be the most important physiologically.[238] MMP stability can be enhanced by MMP- binding proteins. For instance, neutrophil-derived lipocalin protects MMP-9 and may worsen prognosis in breast and gastric cancer.[239,240] MMP activity is inhibited by the endogenous inhibitors TIMPs, but glycosylation status co-determines affinity for TIMP and activity of the MMP.[241] General antiproteinases such as α2-macroglobulin also inhibit MMPs and the resulting inhibitor-MMP complex is subsequently removed from the circulation by scavenger receptors on macrophages. MMPs undergo further autocatalysis, inactivating themselves. Uptake of soluble MMPs by cells is mediated by the LRP receptor followed by degradation in lysosomal vesicles.[198] MT-MMPs are internalized by dynamin-dependent endocytosis in clathrin-coated pits.[242,243]

Matrix metalloproteinases - substrate specificity

A whole array of structural matrix proteins including, but not limited to, collagen I-XI, proteoglycans, elastin, laminin, vitronectin, tenascin, entactin and fibronectin can be cleaved by one or more members of the MMP family. In addition, non-structural proteins such as cytokines, growth hormones and binding proteins (for instance: IL-8, TGF-β and IGFBP-3) are cleaved as well (Table 1).[244] MMPs may also act intracellularly, targeting myosin light chain and troponin in cardiac myocytes.[245,246] Although overlapping, every MMP is characterized by its own substrate specificity, determined by the size and shape of the substrate-binding pocket. For instance, the gelatinases preferentially cleave collagen IV and gelatin,

whereas MMP-1 and -8 preferentially convert collagen I and III. Importantly, most substrate specificities have been determined *in vitro* and remain to be confirmed *in vivo*. In addition, MMPs may act using a non-proteolytic mechanism. For example, binding of TIMP-2 to MMP-14 upregulates cell migration and proliferation by activation of ERK1/-2, a process mediated by the cytoplasmic tail of MMP-14 and not dependent on extracellular proteolytic activity.[247]

Tissue inhibitors of metalloproteinases

The four different TIMPs currently known in humans inhibit activated MMPs by forming non-covalent 1:1 stoichiometric complexes that are resistant to heat-denaturation and proteolytic degradation. TIMP-1 and TIMP-3 also inhibit members of the ADAM and/or ADAMTS family.[248] TIMPs also bind to the proform of MMP-2 and MMP-9, thus regulating the activation process of these MMP members. Different TIMPs have different MMP binding specificities, for instance, TIMP-1 binds preferentially (pro-) MMP-9 but not MMP-2 while TIMP-2 binds (pro-) MMP-2 and not MMP-9. TIMPs are expressed by a variety of cell types including fibroblasts, enterocytes and leucocytes. Expression may be regulated by several cytokines, growth factors, hormones, etc., or is constitutive instead, dependent on TIMP and cell type studied, similar to the regulation of MMP expression. Of note, cytokines (i.e., TGF-β) that repress MMP expression, may enhance levels of TIMP and collagen, promoting a fibrotic phenotype.[249] Expression is also dependent on DNA-methylation and histone-acetylation status.[250] Hypomethylation of the TIMP-1 promoter may result in TIMP-1 expression from the otherwise inactive X chromosome in females, resulting in an overall increase of TIMP-1 levels.[251,252] Conversely, hypermethylation of a TIMP-2 CpG island upstream of the transcription start site is associated with diminished TIMP-2 expression in cervical carcinoma.[253] TIMP-1 and -2 were originally identified as erythroid potentiating factors and it now appears TIMPs are more generally involved in cell growth and/or apoptosis. The TIMP effect may be anti-apoptotic through ligation of the CD63/integrin-β1 complex but also pro-apoptotic via inhibition of MMP-mediated degradation of cell death receptor.[254] Several mutations in the TIMP-3 gene introducing an extra cysteine-residue and promoting dimerization, are associated with Sorsby's fundus dystrophy

and probably other degenerative retinopathies.[255]

Matrix metalloproteinases and tissue inhibitors of metalloproteinases - expression in IBD

MMPs are involved in normal physiological processes where matrix turnover is important, such as wound healing, embryogenesis, angiogenesis, etc. They are also implicated in several disease pathologies such as arthritis, dental disease and cancer metastasis. In IBD, high levels of proinflammatory cytokines in inflamed ulcerated tissue are associated with aberrant expression of MMPs and also TIMPs, but the balance between MMPs and TIMPs appears shifted to a more proteolytic phenotype.[256-262] The increased MMP/TIMP ratio in IBD may result in excessive tissue breakdown and facilitate leucocyte extravasation and migration, although MMP-specific substrate cleavages in IBD mucosa have not been detected so far.[263] Excessive expression of MMPs may also enhance fibroblast trans migration, promoting fibrosis and stenosis, especially in CD.[264-266] Alternatively, the MMP over TIMP ratio may not be sufficiently enhanced to compensate for the increased collagen production by IBD fibroblasts, again resulting in fibrosis.[267] Targeting TIMP-1 with non-functional MMP-9 mutants inhibited liver fibrogenesis, in favor of the second hypothesis.[268] MMPs may also generate new epitopes by cleaving substrates, thus perpetuating the immune response.[269] In several IBD models, administration of synthetic MMP inhibitors improved disease course and DSS-induced colitis was attenuated significantly in MMP-9 deficient mice.[270-272] However, ablation of MMP-2 was observed to aggravate experimental colitis, demonstrating protective capacities of MMPs in IBD as well.[273]

Outline of the studies described in this thesis

Inflammatory bowel disease is of major concern in industrialized countries. Health and economical costs have prompted the initiation of studies aimed at revealing the epidemiology, etiology and pathogenesis of IBD. Both CD and UC are characterized by excessive tissue breakdown during inflammation. The matrix metalloproteinases are important in normal physiological and pathological tissue remodeling and repair processes, including IBD. A short overview of IBD and MMPs is given in **chapter 1**.

Several studies have documented on the altered expression of MMPs in IBD tissue. **Chapter 2** reports on the expression of MMP-2 and MMP-9 in IBD inflamed versus non-inflamed IBD and control intestinal mucosa. MMP levels were measured by enzyme linked immunosorbent assay (ELISA), zymography, activity assay and reverse transcription polymerase (RT-PCR) assay. The cellular localization of MMP expression was determined by immunohistochemistry.

Infliximab is administered to steroid refractory CD patients and targets TNF-α, disrupting proinflammatory communication and promoting apoptosis in leucocytes via reverse signaling. The effect of infliximab on MMP-1, -2, -3, -9 and TIMP-1, -2 protein expression is described in **chapter 3**. Intestinal explants were cultured *ex vivo* with/without (w/wo) infliximab and relative expression of MMP, TIMP, TNF-α was measured by ELISA, activity assay and/or RT-PCR. In addition, explants were cultured w/wo pokeweed mitogen (PWM), to study the expression profiles under inflammatory conditions.

Chapter 4 describes the MMP-2 and MMP-9 serological and mucosal expression profile after the administration of infliximab to CD patients with fistulizing or active disease. Whole blood cultures w/wo infliximab and/or lipopolysaccharide (LPS) were performed to study *in vitro* the contribution of TNF-α in the regulation of MMP-2 and MMP-9 mRNA and protein expression.

In **chapter 5** expression of MMP-1, -2, -3, -9 and TIMP-1, -2 as measured by ELISA and/or activity assays in a large collection of IBD and control intestinal mucosa and related to CD phenotype is reported. The net MMP activity was compared to MMP over TIMP ratio and correlated with myeloperoxidase (MPO) content.

Single nucleotide polymorphisms (SNP) in genes may affect mRNA transcription, stability and/or protein function, thus enhancing disease susceptibility and/or phenotype. **Chapter 6** documents the distribution of (functional) SNPs in the genes encoding MMP-1, -2, -3 and -9, TIMP-1, -2 and TNF-α in a large cohort of IBD patients versus control subjects. Results were correlated with protein expression and clinical course, i.e., development of fistulae, stenotic complications and organ involvement.

After surgical resection, CD patients often experience recurrence of disease. Numerous studies have attempted to identify causal factors and smoking has been established as a bad prognostic factor. In **chapter 7** the clinical course of a large cohort of fully documented CD patients was related to MMP and TIMP genotypes and protein levels in surgically resected intestinal mucosa. In addition, several clinical and demographic variables such as smoking habits, sex and age at resection were retrospectively collected and related to the clinical outcome as well. The different studies are finally compiled as a summarizing discussion in **chapter 8**.

Table 1 (next pages). MMPs and TIMPs, adapted from references.[191,192,244,274] Pre = prepeptide signal sequence, pro = propeptide, catalytic = catalytic domain, Zn = catalytic zinc, F = fibronectin type II repeat, Fu = furin pro-protein convertase cleavage site, TM = transmembrane region, cyt = cytoplasmic tail, GPI= glycosylphosphatidylinositol anchor, CA = cysteine array, Ig = immunoglobulin, V= vitronectin insert. Note: substrate specificities were determined *in vitro* and remain to be confirmed *in vivo*. MMPs may digest other substrates not mentioned in this overview. *Cellular expression is dependent on stimulation by cytokines, extracellular matrix, DNA-methylation and acetylation, oncogenic transformation, etc., and expression should not be viewed as limited to those cells or tissues mentioned. References indicated between brackets.

Table 1.

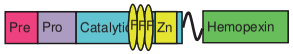
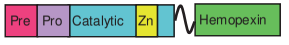
Subgroup	MMP	Trivial name	Chromo-somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificiy	Cellular source*
Collagenases	MMP-1	Interstitial collagenase; collagenase 1	11q22-q23		Collagen I, II, III, VII, X, gelatin, entactin, aggrecan, tenascin, perlecan	IGFBP-2,3 Pro-MMP-1,2; pro-TNF-α, α1-antichymotrypsin, α1-proteinase inhibitor	Fibroblasts, keratinocytes, macrophages [275-277]
	MMP-8	Neutrophil collagenase; collagenase 2	11q21-q22			Pro-MMP-8, α1-proteinase inhibitor	Neutrophils, fibroblasts [278,279]
	MMP-13	Collagenase 3	11q22.3			Pro-MMP-9, 13; α1-antichymotrypsin	Chondrocytes, osteoblasts [280,281]
Gelatinases	MMP-2	Gelatinase A	16q13		Gelatin, elastin, fibronectin, collagen I/IV/V/VII/X/XI, laminin, aggrecan, vitronectin	Decorin, pro-TGF-β2, pro-IL-1β, MCP-3, IGFBP-3/5, pro-TNF-α, FGF-R1, pro-MMP-1, 2, 13	Fibroblasts, vascular smooth muscle cells, T lymphocytes [282-285]
	MMP-9	Gelatinase B	20q11.2-q13.1			Pro-TGF-β2, pro-IL-1β, IL-8, cell surface bound IL-2Ra, plasminogen, α1-proteinase inhibitor, pro-TNF-α	Neutrophils, monocytes/ macrophages, Fibroblasts [286-288]
Stromelysins	MMP-3	Stromelysin 1	11q23		Proteoglycans, laminin, fibronectin, gelatin, collagen III/IV/V/IX/X/XI, fibrin/fibrinogen, entactin, tenascin, vitronectin	Perlecan, decorin, pro-HB-EGF, pro-IL-1β, plasminogen, E-cadherin, IGFBP-3, α1-antichymotrypsin, α1-proteinase inhibitor, pro-MMP-1, 3, 7, 8, 9, 13, pro-TNF-α	Fibroblasts, chondrocytes, vascular smooth muscle cells, keratinocytes, macrophages and endothelial cells [289-294]
	MMP-10	Stromelysin 2	11q22.3-q23			Pro-MMP-1, 8, 10	Keratinocytes, monocytes, fibroblasts [294-296]

Table 1, continued.

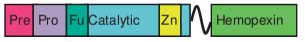
Subgroup	MMP	Trivial name	Chromo-somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificiy	Cellular source*
Matrilysins	MMP-7	Matrilysin 1	11q21-q22			Proteoglycans, laminin, fibronectin, gelatin, collagen III, IV, V, IX, X, XI, fibrin/fibrinogen, entactin, tenascin, vitronectin, pro-α-defensin, decorin, cell surface bound FasL, β4-integrin, E-cadherin, plasminogen, pro-TNF-α, pro-MMP-2, 7	Epithelial cells [297,298]
	MMP-26	Matrilysin 2	11p15			Gelatin, collagen IV, fibronectin, fibrinogen, α1-proteinase inhibitor, MMP-9	Placenta, uterus, ovary, keratinocytes [299-301]
	MMP-11	Stromelysin 3	22q11.2			Laminin, fibronectin, aggrecan, α1- proteinase inhibitor, IGFBP-1	Fibroblasts, B-lymphocytes [295,302]
Membrane type MMPs -trans-membrane type	MMP-14	MT1-MMP	14q11-q12		Gelatin, fibronectin, vitronectin, collagen, aggrecan	Pro-MMP-2, 13; cell surface bound CD44, cell surface bound tissue transglutaminase (tTG)	Endothelial cells, hepatic stellate cells, fibroblasts [303-305]
	MMP-15	MT2-MMP	15q13-q21			TTG	Central Nervous system microglia [306]
	MMP-16	MT3-MMP	8q21			Pro-MMP-2, tTG	Chondrocytes [307]
	MMP-24	MT5-MMP	20q11.2			Pro-MMP-2	T-lymphocytes, fibroblasts [277,295]

Table 1, continued.

Subgroup	MMP	Trivial name	Chromo-somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificiy	Cellular source*
Membrane type MMPs -GPI anchored	MMP-17	MT4-MMP	12q24.3			Gelatin, pro-MMP-2	Monocytes, B-lymphocytes [295]
	MMP-25	MT6-MMP	16p13.3			Gelatin, collagen IV, fibrin, fibronectin, laminin-1, pro-MMP-2	Monocytes, neutrophils [229,295]
Others	MMP-12	Macrophage elastase	11q22.2-q22.3			Elastin, fibronectin, fibrin, fibrinogen, laminin, proteoglycan, plasminogen	Macrophages [308]
	MMP-19	-	12q14			Gelatin, tenascin, fibronectin, collagen IV, laminin, entactin, fibrin/fibrinogen, aggrecan, COMP	Keratinocytes, brain microglia [309,310]
	MMP-20	Enamelysin	11q22.3			Amelogenin, aggrecan, COMP	Tooth enamel forming ameloblasts (epithelial origin) [311]
	MMP-21	-	?			Gelatin	Keratinocytes [312]
	MMP-23	CA-MMP	1p36.3			Gelatin	Reproductive organs [313]
	MMP-27	-	11q24				B-lymphocytes [295]
	MMP-28	Epilysin	17q21.1				Keratinocytes, intestine, T-lymphocytes [257,295,314]

References

1. Herszenyi L, Miheller P, Tulassay Z. Carcinogenesis in inflammatory bowel disease. *Dig Dis.* 2007;25:267-69.
2. Jess T, Gamborg M, Matzen P, *et al*. Increased risk of intestinal cancer in Crohn's disease: a meta-analysis of population-based cohort studies. *Am J Gastroenterol.* 2005;100:2724-29.
3. Cornish J, Tan E, Teare J, *et al*. A meta-analysis on the influence of inflammatory bowel disease on pregnancy. *Gut.* 2007;56:830-837.
4. Osterman MT, Lichtenstein GR. Infliximab in fistulizing Crohn's disease. *Gastroenterol Clin North Am.* 2006;35:795-820.
5. Plosker GL, Lyseng-Williamson KA. Adalimumab: in Crohn's disease. *BioDrugs.* 2007;21:125-32.
6. Richter JA, Bickston SJ. Infliximab use in luminal Crohn's disease. *Gastroenterol Clin North Am.* 2006;35:775-93.
7. Jewell DP, Satsangi J, Lobo A, *et al*. Infliximab use in Crohn's disease: impact on health care resources in the UK. *Eur J Gastroenterol Hepatol.* 2005;17:1047-52.
8. Ollendorf DA, Lidsky L. Infliximab drug and infusion costs among patients with Crohn's disease in a commercially-insured setting. *Am J Ther.* 2006;13:502-6.
9. D'haens G, Daperno M. Advances in biologic therapy for ulcerative colitis and Crohn's disease. *Curr Gastroenterol Rep.* 2006;8:506-12.
10. Van Assche G. Emerging drugs to treat Crohn's disease. Expert Opin Emerg *Drugs.* 2007;12:49-59.
11. Vermeire S, Van Assche G, Rutgeerts P. Review article: Altering the natural history of Crohn's disease--evidence for and against current therapies. *Aliment Pharmacol Ther.* 2007;25:3-12.
12. Wolters FL, Russel MG, Stockbrugger RW. Systematic review: has disease outcome in Crohn's disease changed during the last four decades? *Aliment Pharmacol Ther.* 2004;20:483-96.
13. Gupta N, Cohen SA, Bostrom AG, *et al*. Risk factors for initial surgery in pediatric patients with Crohn's disease. *Gastroenterology.* 2006;130:1069-77.

14. Jess T, Riis L, Vind I, *et al*. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. *Inflamm Bowel Dis.* 2007;13:481-89.
15. Loftus EV, Jr., Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. *Aliment Pharmacol Ther.* 2002;16:51-60.
16. Vind I, Riis L, Jess T, *et al*. Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003-2005: a population-based study from the Danish Crohn colitis database. *Am J Gastroenterol.* 2006;101:1274-82.
17. Sandler RS, Everhart JE, Donowitz M, *et al*. The burden of selected digestive diseases in the United States. *Gastroenterology.* 2002;122:1500-1511.
18. Longobardi T, Jacobs P, Bernstein CN. Work losses related to inflammatory bowel disease in the United States: results from the National Health Interview Survey. *Am J Gastroenterol.* 2003;98:1064-72.
19. Fonager K, Sorensen HT, Olsen J. Change in incidence of Crohn's disease and ulcerative colitis in Denmark. A study based on the National Registry of Patients, 1981-1992. *Int J Epidemiol.* 1997;26:1003-8.
20. Loftus CG, Loftus EV, Jr., Harmsen WS, *et al*. Update on the incidence and prevalence of Crohn's disease and ulcerative colitis in Olmsted County, Minnesota, 1940-2000. *Inflamm Bowel Dis.* 2007;13:254-61.
21. Russel MG, Dorant E, Volovics A, *et al*. High incidence of inflammatory bowel disease in The Netherlands: results of a prospective study. The South Limburg IBD Study Group. *Dis Colon Rectum.* 1998;41:33-40.
22. Stone MA, Mayberry JF, Baker R. Prevalence and management of inflammatory bowel disease: a cross-sectional study from central England. *Eur J Gastroenterol Hepatol.* 2003;15:1275-80.
23. Bernstein CN, Blanchard JF, Rawsthorne P, *et al*. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am J Epidemiol.* 1999;149:916-24.

24. Gearry RB, Richardson A, Frampton CM, *et al*. High incidence of Crohn's disease in Canterbury, New Zealand: results of an epidemiologic study. *Inflamm Bowel Dis.* 2006;12:936-43.
25. Loftus EV, Jr., Silverstein MD, Sandborn WJ, *et al*. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology.* 1998;114:1161-68.
26. Loftus EV, Jr., Silverstein MD, Sandborn WJ, *et al*. Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gut.* 2000;46:336-43.
27. Sonnenberg A. Occupational distribution of inflammatory bowel disease among German employees. *Gut.* 1990;31:1037-40.
28. Shivananda S, Lennard-Jones J, Logan R, *et al*. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut.* 1996;39:690-697.
29. Ouyang Q, Tandon R, Goh KL, *et al*. The emergence of inflammatory bowel disease in the Asian Pacific region. *Curr Opin Gastroenterol.* 2005;21:408-13.
30. Tsironi E, Feakins RM, Probert CS, *et al*. Incidence of inflammatory bowel disease is rising and abdominal tuberculosis is falling in Bangladeshis in East London, United Kingdom. *Am J Gastroenterol.* 2004;99:1749-55.
31. Orholm M, Binder V, Sorensen TI, *et al*. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol.* 2000;35:1075-81.
32. Yamamoto T. Factors affecting recurrence after surgery for Crohn's disease. *World J Gastroenterol.* 2005;11:3971-79.
33. Hoie O, Wolters F, Riis L, *et al*. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. *Am J Gastroenterol.* 2007;102:1692-701.
34. Feller M, Huwiler K, Stephan R, *et al*. Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. *Lancet Infect Dis.* 2007;7:607-13.

35. Darfeuille-Michaud A, Boudeau J, Bulois P, *et al*. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology.* 2004;127:412-21.
36. Sasaki M, Sitaraman SV, Babbin BA, *et al*. Invasive Escherichia coli are a feature of Crohn's disease. *Lab Invest.* 2007;87:1042-54.
37. Morris DL, Montgomery SM, Thompson NP, *et al*. Measles vaccination and inflammatory bowel disease: a national British Cohort Study. *Am J Gastroenterol.* 2000;95:3507-12.
38. Pardi DS, Tremaine WJ, Sandborn WJ, *et al*. Early measles virus infection is associated with the development of inflammatory bowel disease. *Am J Gastroenterol.* 2000;95:1480-1485.
39. Thompson NP, Montgomery SM, Pounder RE, *et al*. Is measles vaccination a risk factor for inflammatory bowel disease? *Lancet.* 1995;345:1071-74.
40. Vare PO, Heikius B, Silvennoinen JA, *et al*. Seroprevalence of Helicobacter pylori infection in inflammatory bowel disease: is Helicobacter pylori infection a protective factor? *Scand J Gastroenterol.* 2001;36:1295-300.
41. Laharie D, Debeugny S, Peeters M, *et al*. Inflammatory bowel disease in spouses and their offspring. *Gastroenterology.* 2001;120:816-19.
42. Chowers Y, Odes S, Bujanover Y, *et al*. The month of birth is linked to the risk of Crohn's disease in the Israeli population. *Am J Gastroenterol*. 2004;99:1974-76.
43. Moum B, Aadland E, Ekbom A, *et al*. Seasonal variations in the onset of ulcerative colitis. *Gut.* 1996;38:376-78.
44. Card TR, Sawczenko A, Sandhu BK, *et al*. No seasonality in month of birth of inflammatory bowel disease cases: a prospective population based study of British under 20 year olds. *Gut.* 2002;51:814-15.
45. Corrao G, Tragnone A, Caprilli R, *et al*. Risk of inflammatory bowel disease attributable to smoking, oral contraception and breastfeeding in Italy: a nationwide case-control study. Cooperative Investigators of the Italian Group for the Study of the Colon and the Rectum (GISC). *Int J Epidemiol*. 1998;27:397-404.

46. Persson PG, Ahlbom A, Hellers G. Diet and inflammatory bowel disease: a case-control study. *Epidemiology.* 1992;3:47-52.
47. Russel MG, Engels LG, Muris JW, *et al*. Modern life' in the epidemiology of inflammatory bowel disease: a case-control study with special emphasis on nutritional factors. *Eur J Gastroenterol Hepatol.* 1998;10:243-49.
48. Garcia Rodriguez LA, Gonzalez-Perez A, Johansson S, *et al*. Risk factors for inflammatory bowel disease in the general population. *Aliment Pharmacol Ther.* 2005;22:309-15.
49. Persson PG, Leijonmarck CE, Bernell O, *et al*. Risk indicators for inflammatory bowel disease. *Int J Epidemiol.* 1993;22:268-72.
50. Kane SV, Sable K, Hanauer SB. The menstrual cycle and its effect on inflammatory bowel disease and irritable bowel syndrome: a prevalence study. *Am J Gastroenterol.* 1998;93:1867-72.
51. Mittermaier C, Dejaco C, Waldhoer T, *et al*. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. *Psychosom Med.* 2004;66:79-84.
52. Mikocka-Walus AA, Turnbull DA, Moulding NT, *et al*. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflamm Bowel Dis.* 2007;13:225-34.
53. Delco F, Sonnenberg A. Military history of patients with inflammatory bowel disease: an epidemiological study among U.S. veterans. *Am J Gastroenterol*. 1998;93:1457-62.
54. Gent AE, Hellier MD, Grace RH, *et al*. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet.* 1994;343:766-67.
55. Green C, Elliott L, Beaudoin C, *et al*. A population-based ecologic study of inflammatory bowel disease: searching for etiologic clues. *Am J Epidemiol*. 2006;164:615-23.
56. Tedeschi A, Airaghi L. Is affluence a risk factor for bronchial asthma and type 1 diabetes? *Pediatr Allergy Immunol.* 2006;17:533-37.
57. Binder V, Orholm M. Familial occurrence and inheritance studies in inflammatory bowel disease. *Neth J Med.* 1996;48:53-56.

58. Kurata JH, Kantor-Fish S, Frankl H, *et al*. Crohn's disease among ethnic groups in a large health maintenance organization. *Gastroenterology.* 1992;102:1940-1948.
59. Roth MP, Petersen GM, McElree C, *et al*. Geographic origins of Jewish patients with inflammatory bowel disease. *Gastroenterology.* 1989;97:900-904.
60. Sonnenberg A, Wasserman IH. Epidemiology of inflammatory bowel disease among U.S. military veterans. *Gastroenterology.* 1991;101:122-30.
61. Hampe J, Schreiber S, Shaw SH, *et al*. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet.* 1999;64:808-16.
62. Hugot JP, Laurent-Puig P, Gower-Rousseau C, *et al*. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature.* 1996;379:821-23.
63. Ma Y, Ohmen JD, Li Z, *et al*. A genome-wide search identifies potential new susceptibility loci for Crohn's disease. *Inflamm Bowel Dis.* 1999;5:271-78.
64. Rioux JD, Silverberg MS, Daly MJ, *et al*. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet.* 2000;66:1863-70.
65. Satsangi J, Parkes M, Louis E, *et al*. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet.* 1996;14:199-202.
66. Vermeire S, Satsangi J, Peeters M, *et al*. Evidence for inflammatory bowel disease of a susceptibility locus on the X chromosome. *Gastroenterology.* 2001;120:834-40.
67. Hugot JP, Chamaillard M, Zouali H, *et al*. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001;411:599-603.
68. Ogura Y, Bonen DK, Inohara N, *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature.* 2001;411:603-6.

69. Brant SR, Wang MH, Rawsthorne P, *et al*. A population-based case-control study of CARD15 and other risk factors in Crohn's disease and ulcerative colitis. *Am J Gastroenterol.* 2007;102:313-23.
70. Cuthbert AP, Fisher SA, Mirza MM, *et al*. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology.* 2002;122:867-74.
71. Weidinger S, Klopp N, Rummler L, *et al*. Association of CARD15 polymorphisms with atopy-related traits in a population-based cohort of Caucasian adults. *Clin Exp Allergy.* 2005;35:866-72.
72. Miceli-Richard C, Lesage S, Rybojad M, *et al*. CARD15 mutations in Blau syndrome. *Nat Genet.* 2001;29:19-20.
73. Brenmoehl J, Herfarth H, Gluck T, *et al*. Genetic variants in the NOD2/CARD15 gene are associated with early mortality in sepsis patients. *Intensive Care Med.* 2007;33:1541-48.
74. Holler E, Rogler G, Herfarth H, *et al*. Both donor and recipient NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. *Blood.* 2004;104:889-94.
75. Mayor NP, Shaw BE, Hughes DA, *et al*. Single nucleotide polymorphisms in the NOD2/CARD15 gene are associated with an increased risk of relapse and death for patients with acute leukemia after hematopoietic stem-cell transplantation with unrelated donors. *J Clin Oncol.* 2007;25:4262-69.
76. Duerr RH, Taylor KD, Brant SR, *et al*. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science.* 2006;314:1461-63.
77. Tremelling M, Cummings F, Fisher SA, *et al*. IL23R variation determines susceptibility but not disease phenotype in inflammatory bowel disease. *Gastroenterology.* 2007;132:1657-64.
78. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007;447:661-78.
79. Hampe J, Franke A, Rosenstiel P, *et al*. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet.* 2007;39:207-11.

80. Parkes M, Barrett JC, Prescott NJ, *et al*. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet.* 2007;39:830-832.
81. Prescott NJ, Fisher SA, Franke A, *et al*. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterology.* 2007;132:1665-71.
82. Rioux JD, Xavier RJ, Taylor KD, *et al*. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet.* 2007;39:596-604.
83. Libioulle C, Louis E, Hansoul S, *et al*. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet.* 2007;3:e58.
84. Franke A, Hampe J, Rosenstiel P, *et al*. Systematic association mapping identifies NELL1 as a novel IBD disease gene. *PLoS ONE.* 2007;2:e691.
85. Franke A, Balschun T, Karlsen TH, *et al*. Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. *Nat Genet.* 2008;40:713-15.
86. Roberts RL, Gearry RB, Hollis-Moffatt JE, *et al*. IL23R R381Q and ATG16L1 T300A Are Strongly Associated With Crohn's Disease in a Study of New Zealand Caucasians With Inflammatory Bowel Disease. *Am J Gastroenterol.* 2007.
87. Yamazaki K, Onouchi Y, Takazoe M, *et al*. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. *J Hum Genet.* 2007;52:575-83.
88. Aithal GP, Day CP, Leathart J, *et al*. Association of single nucleotide polymorphisms in the interleukin-4 gene and interleukin-4 receptor gene with Crohn's disease in a British population. *Genes Immun.* 2001;2:44-47.
89. Dring MM, Goulding CA, Trimble VI, *et al*. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology.* 2006;130:341-48.
90. Franchimont D, Vermeire S, El Housni H, *et al*. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4

Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut.* 2004;53:987-92.

91. Ho GT, Nimmo ER, Tenesa A, *et al*. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology.* 2005;128:288-96.
92. Karban AS, Okazaki T, Panhuysen CI, *et al*. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. *Hum Mol Genet.* 2004;13:35-45.
93. Klein W, Tromm A, Griga T, *et al*. Interleukin-4 and interleukin-4 receptor gene polymorphisms in inflammatory bowel diseases. *Genes Immun.* 2001;2:287-89.
94. Klein W, Tromm A, Griga T, *et al*. A polymorphism in the IL11 gene is associated with ulcerative colitis. *Genes Immun.* 2002;3:494-96.
95. Kyo K, Parkes M, Takei Y, *et al*. Association of ulcerative colitis with rare VNTR alleles of the human intestinal mucin gene, MUC3. *Hum Mol Genet.* 1999;8:307-11.
96. Mansfield JC, Holden H, Tarlow JK, *et al*. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology.* 1994;106:637-42.
97. Moehle C, Ackermann N, Langmann T, *et al*. Aberrant intestinal expression and allelic variants of mucin genes associated with inflammatory bowel disease. *J Mol Med.* 2006;84:1055-66.
98. Parkes M, Satsangi J, Jewell D. Contribution of the IL-2 and IL-10 genes to inflammatory bowel disease (IBD) susceptibility. *Clin Exp Immunol.* 1998;113:28-32.
99. Peltekova VD, Wintle RF, Rubin LA, *et al*. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet.* 2004;36:471-75.
100. Pierik M, Joossens S, Van Steen K, *et al*. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2006;12:1-8.

101. Rector A, Lemey P, Laffut W, *et al*. Mannan-binding lectin (MBL) gene polymorphisms in ulcerative colitis and Crohn's disease. *Genes Immun.* 2001;2:323-28.
102. Stoll M, Corneliussen B, Costello CM, *et al*. Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet.* 2004;36:476-80.
103. Hacker UT, Gomolka M, Keller E, *et al*. Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. *Gut.* 1997;40:623-27.
104. Noble CL, Nimmo ER, Drummond H, *et al*. DLG5 variants do not influence susceptibility to inflammatory bowel disease in the Scottish population. *Gut.* 2005;54:1416-20.
105. Oostenbrug LE, Dijkstra G, Nolte IM, *et al*. Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. *Scand J Gastroenterol.* 2006;41:1174-82.
106. Zipperlen K, Peddle L, Melay B, *et al*. Association of TNF-alpha polymorphisms in Crohn disease. *Hum Immunol.* 2005;66:56-59.
107. Kontoyiannis D, Pasparakis M, Pizarro TT, *et al*. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity.* 1999;10:387-98.
108. Kuhn R, Lohler J, Rennick D, *et al*. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell.* 1993;75:263-74.
109. Mombaerts P, Mizoguchi E, Grusby MJ, *et al*. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell.* 1993;75:274-82.
110. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol.* 1998;161:5733-44.
111. Sadlack B, Merz H, Schorle H, *et al*. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell.* 1993;75:253-61.
112. Shull MM, Ormsby I, Kier AB, *et al*. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature.* 1992;359:693-99.

113. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest.* 2007;117:514-21.
114. Hugot JP, Alberti C, Berrebi D, *et al*. Crohn's disease: the cold chain hypothesis. *Lancet.* 2003;362:2012-15.
115. Mei L, Targan SR, Landers CJ, *et al*. Familial expression of anti-Escherichia coli outer membrane porin C in relatives of patients with Crohn's disease. *Gastroenterology.* 2006;130:1078-85.
116. Stevens TR, Winrow VR, Blake DR, *et al*. Circulating antibodies to heat-shock protein 60 in Crohn's disease and ulcerative colitis. *Clin Exp Immunol.* 1992;90:271-74.
117. Targan SR, Landers CJ, Yang H, *et al*. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology.* 2005;128:2020-2028.
118. Lodes MJ, Cong Y, Elson CO, *et al*. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest.* 2004;113:1296-306.
119. Bamias G, Okazawa A, Rivera-Nieves J, *et al*. Commensal bacteria exacerbate intestinal inflammation but are not essential for the development of murine ileitis. *J Immunol.* 2007;178:1809-18.
120. Wehkamp J, Harder J, Weichenthal M, *et al*. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut.* 2004;53:1658-64.
121. Buisine MP, Desreumaux P, Debailleul V, *et al*. Abnormalities in mucin gene expression in Crohn's disease. *Inflamm Bowel Dis.* 1999;5:24-32.
122. Zeissig S, Burgel N, Gunzel D, *et al*. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut.* 2007;56:61-72.
123. Marks DJ, Harbord MW, MacAllister R, *et al*. Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet.* 2006;367:668-78.
124. Boirivant M, Marini M, Di Felice G, *et al*. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology.* 1999;116:557-65.

125. Brannigan AE, O'Connell PR, Hurley H, *et al*. Neutrophil apoptosis is delayed in patients with inflammatory bowel disease. *Shock.* 2000;13:361-66.
126. Fayad R, Brand MI, Stone D, *et al*. Apoptosis resistance in ulcerative colitis: high expression of decoy receptors by lamina propria T cells. *Eur J Immunol.* 2006;36:2215-22.
127. Itoh J, de La MC, Strong SA, *et al*. Decreased Bax expression by mucosal T cells favours resistance to apoptosis in Crohn's disease. *Gut.* 2001;49:35-41.
128. Maul J, Loddenkemper C, Mundt P, *et al*. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. *Gastroenterology.* 2005;128:1868-78.
129. Andus T, Daig R, Vogl D, *et al*. Imbalance of the interleukin 1 system in colonic mucosa--association with intestinal inflammation and interleukin 1 receptor antagonist [corrected] genotype 2. *Gut.* 1997;41:651-57.
130. Autschbach F, Schurmann G, Qiao L, *et al*. Cytokine messenger RNA expression and proliferation status of intestinal mononuclear cells in noninflamed gut and Crohn's disease. *Virchows Arch.* 1995;426:51-60.
131. Banks C, Bateman A, Payne R, *et al*. Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease. *J Pathol.* 2003;199:28-35.
132. Bernstein CN, Sargent M, Rector E. Alteration in expression of beta 2 integrins on lamina propria lymphocytes in ulcerative colitis and Crohn's disease. *Clin Immunol.* 2002;104:67-72.
133. Brauchle M, Madlener M, Wagner AD, *et al*. Keratinocyte growth factor is highly overexpressed in inflammatory bowel disease. *Am J Pathol.* 1996;149:521-29.
134. Brynskov J, Tvede N, Andersen CB, *et al*. Increased concentrations of interleukin 1 beta, interleukin-2, and soluble interleukin-2 receptors in endoscopical mucosal biopsy specimens with active inflammatory bowel disease. *Gut.* 1992;33:55-58.
135. Cui G, Olsen T, Christiansen I, *et al*. Improvement of real-time polymerase chain reaction for quantifying TNF-alpha mRNA expression in inflamed

colorectal mucosa: an approach to optimize procedures for clinical use. *Scand J Clin Lab Invest.* 2006;66:249-59.

136. Daig R, Andus T, Aschenbrenner E, *et al*. Increased interleukin 8 expression in the colon mucosa of patients with inflammatory bowel disease. *Gut.* 1996;38:216-22.
137. Fujino S, Andoh A, Bamba S, *et al*. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut.* 2003;52:65-70.
138. Griga T, May B, Pfisterer O, *et al*. Immunohistochemical localization of vascular endothelial growth factor in colonic mucosa of patients with inflammatory bowel disease. *Hepatogastroenterology.* 2002;49:116-23.
139. Gustot T, Lemmers A, Louis E, *et al*. Profile of soluble cytokine receptors in Crohn's disease. *Gut.* 2005;54:488-95.
140. Kanazawa S, Tsunoda T, Onuma E, *et al*. VEGF, basic-FGF, and TGF-beta in Crohn's disease and ulcerative colitis: a novel mechanism of chronic intestinal inflammation. *Am J Gastroenterol.* 2001;96:822-28.
141. Kaser A, Ludwiczek O, Holzmann S, *et al*. Increased expression of CCL20 in human inflammatory bowel disease. *J Clin Immunol.* 2004;24:74-85.
142. McAlindon ME, Hawkey CJ, Mahida YR. Expression of interleukin 1 beta and interleukin 1 beta converting enzyme by intestinal macrophages in health and inflammatory bowel disease. *Gut.* 1998;42:214-19.
143. McCormack G, Moriarty D, O'Donoghue DP, *et al*. Tissue cytokine and chemokine expression in inflammatory bowel disease. *Inflamm Res.* 2001;50:491-95.
144. Monteleone G, Mann J, Monteleone I, *et al*. A failure of transforming growth factor-beta1 negative regulation maintains sustained NF-kappaB activation in gut inflammation. *J Biol Chem.* 2004;279:3925-32.
145. Nakamura S, Ohtani H, Watanabe Y, *et al*. *In situ* expression of the cell adhesion molecules in inflammatory bowel disease. Evidence of immunologic activation of vascular endothelial cells. *Lab Invest.* 1993;69:77-85.
146. Noguchi M, Hiwatashi N, Liu Z, *et al*. Secretion imbalance between tumour necrosis factor and its inhibitor in inflammatory bowel disease. *Gut.* 1998;43:203-9.

147. Reimund JM, Wittersheim C, Dumont S, *et al*. Increased production of tumour necrosis factor-alpha interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. *Gut.* 1996;39:684-89.
148. Sakai T, Kusugami K, Nishimura H, *et al*. Interleukin 15 activity in the rectal mucosa of inflammatory bowel disease. *Gastroenterology.* 1998;114:1237-43.
149. Schmidt C, Baumeister B, Kipnowski J, *et al*. Alteration of prostaglandin E2 and leukotriene B4 synthesis in chronic inflammatory bowel disease. *Hepatogastroenterology.* 1996;43:1508-12.
150. Schreiber S, Heinig T, Thiele HG, *et al*. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology.* 1995;108:1434-44.
151. Seegert D, Rosenstiel P, Pfahler H, *et al*. Increased expression of IL-16 in inflammatory bowel disease. *Gut.* 2001;48:326-32.
152. Shioya M, Nishida A, Yagi Y, *et al*. Epithelial overexpression of interleukin-32alpha in inflammatory bowel disease. *Clin Exp Immunol.* 2007;149:480-486.
153. Souza HS, Elia CC, Spencer J, *et al*. Expression of lymphocyte-endothelial receptor-ligand pairs, alpha4beta7/MAdCAM-1 and OX40/OX40 ligand in the colon and jejunum of patients with inflammatory bowel disease. *Gut.* 1999;45:856-63.
154. ter Beek WP, Biemond I, Muller ES, *et al*. Substance P receptor expression in patients with inflammatory bowel disease. Determination by three different techniques, i.e., storage phosphor autoradiography, RT-PCR and immunohistochemistry. *Neuropeptides.* 2007;41:301-6.
155. Woywodt A, Ludwig D, Neustock P, *et al*. Mucosal cytokine expression, cellular markers and adhesion molecules in inflammatory bowel disease. *Eur J Gastroenterol Hepatol.* 1999;11:267-76.
156. Holtmann MH, Douni E, Schutz M, *et al*. Tumor necrosis factor-receptor 2 is up-regulated on lamina propria T cells in Crohn's disease and promotes experimental colitis *in vivo*. *Eur J Immunol.* 2002;32:3142-51.

157. Heller F, Florian P, Bojarski C, *et al*. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology.* 2005;129:550-564.
158. McClane SJ, Rombeau JL. Cytokines and inflammatory bowel disease: a review. *JPEN J Parenter Enteral Nutr.* 1999;23:S20-S24.
159. Monteleone G, Biancone L, Marasco R, *et al*. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology.* 1997;112:1169-78.
160. Neurath MF. IL-23: a master regulator in Crohn disease. *Nat Med.* 2007;13:26-28.
161. Brandt E, Colombel JF, Ectors N, *et al*. Enhanced production of IL-8 in chronic but not in early ileal lesions of Crohn's disease (CD). *Clin Exp Immunol.* 2000;122:180-185.
162. Desreumaux P, Brandt E, Gambiez L, *et al*. Distinct cytokine patterns in early and chronic ileal lesions of Crohn's disease. *Gastroenterology.* 1997;113:118-26.
163. D'Inca R, Cardin R, Benazzato L, *et al*. Oxidative DNA damage in the mucosa of ulcerative colitis increases with disease duration and dysplasia. *Inflamm Bowel Dis.* 2004;10:23-27.
164. Keshavarzian A, Banan A, Farhadi A, *et al*. Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut.* 2003;52:720-728.
165. Kruidenier L, Kuiper I, Lamers CB, *et al*. Intestinal oxidative damage in inflammatory bowel disease: semi-quantification, localization, and association with mucosal antioxidants. *J Pathol.* 2003;201:28-36.
166. Nair J, Gansauge F, Beger H, *et al*. Increased etheno-DNA adducts in affected tissues of patients suffering from Crohn's disease, ulcerative colitis, and chronic pancreatitis. *Antioxid Redox Signal.* 2006;8:1003-10.
167. Kruidenier L, Kuiper I, van Duijn W, *et al*. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. *J Pathol.* 2003;201:17-27.

168. Kruidenier L, Kuiper I, van Duijn W, *et al*. Differential mucosal expression of three superoxide dismutase isoforms in inflammatory bowel disease. *J Pathol.* 2003;201:7-16.
169. Tamaki H, Nakamura H, Nishio A, *et al*. Human thioredoxin-1 ameliorates experimental murine colitis in association with suppressed macrophage inhibitory factor production. *Gastroenterology.* 2006;131:1110-1121.
170. Jenkins D, Seth R, Kummer JA, *et al*. Differential levels of granzyme B, regulatory cytokines, and apoptosis in Crohn's disease and ulcerative colitis at first presentation. *J Pathol.* 2000;190:184-89.
171. Souza HS, Tortori CJ, Castelo-Branco MT, *et al*. Apoptosis in the intestinal mucosa of patients with inflammatory bowel disease: evidence of altered expression of FasL and perforin cytotoxic pathways. *Int J Colorectal Dis.* 2005;20:277-86.
172. Ueyama H, Kiyohara T, Sawada N, *et al*. High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut.* 1998;43:48-55.
173. Allez M, Tieng V, Nakazawa A, *et al*. CD4+NKG2D+ T cells in Crohn's disease mediate inflammatory and cytotoxic responses through MICA interactions. *Gastroenterology.* 2007;132:2346-58.
174. Muller S, Lory J, Corazza N, *et al*. Activated CD4+ and CD8+ cytotoxic cells are present in increased numbers in the intestinal mucosa from patients with active inflammatory bowel disease. *Am J Pathol.* 1998;152:261-68.
175. Ebert EC, Geng X, Lin J, *et al*. Autoantibodies against human tropomyosin isoform 5 in ulcerative colitis destroys colonic epithelial cells through antibody and complement-mediated lysis. *Cell Immunol.* 2006;244:43-49.
176. Halstensen TS, Das KM, Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kD putative autoantigen in ulcerative colitis. *Gut.* 1993;34:650-657.
177. Andoh A, Deguchi Y, Inatomi O, *et al*. Immunohistochemical study of chymase-positive mast cells in inflammatory bowel disease. *Oncol Rep.* 2006;16:103-7.

178. Raithel M, Winterkamp S, Pacurar A, *et al*. Release of mast cell tryptase from human colorectal mucosa in inflammatory bowel disease. *Scand J Gastroenterol.* 2001;36:174-79.
179. Saitoh O, Sugi K, Matsuse R, *et al*. The forms and the levels of fecal PMN-elastase in patients with colorectal diseases. *Am J Gastroenterol.* 1995;90:388-93.
180. Isozaki Y, Yoshida N, Kuroda M, *et al*. Anti-tryptase treatment using nafamostat mesilate has a therapeutic effect on experimental colitis. *Scand J Gastroenterol.* 2006;41:944-53.
181. Morohoshi Y, Matsuoka K, Chinen H, *et al*. Inhibition of neutrophil elastase prevents the development of murine dextran sulfate sodium-induced colitis. *J Gastroenterol.* 2006;41:318-24.
182. Schmid M, Fellermann K, Fritz P, *et al*. Attenuated induction of epithelial and leukocyte serine antiproteases elafin and secretory leukocyte protease inhibitor in Crohn's disease. *J Leukoc Biol.* 2007;81:907-15.
183. Rawlings ND, Morton FR, Barrett AJ. MEROPS: the peptidase database. *Nucleic Acids Res.* 2006;34:D270-D272.
184. Bode W, Gomis-Ruth FX, Stockler W. Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. *FEBS Lett.* 1993;331:134-40.
185. Blobel CP. ADAMs: key components in EGFR signalling and development. *Nat Rev Mol Cell Biol.* 2005;6:32-43.
186. Jones GC, Riley GP. ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. *Arthritis Res Ther.* 2005;7:160-169.
187. Bell JH, Herrera AH, Li Y, *et al*. Role of ADAM17 in the ectodomain shedding of TNF-alpha and its receptors by neutrophils and macrophages. *J Leukoc Biol.* 2007;82:173-76.
188. Gendron C, Kashiwagi M, Lim NH, *et al*. Proteolytic activities of human ADAMTS-5: comparative studies with ADAMTS-4. *J Biol Chem.* 2007;282:18294-306.

189. Song RH, Tortorella MD, Malfait AM, *et al*. Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum.* 2007;56:575-85.
190. Stanton H, Rogerson FM, East CJ, *et al*. ADAMTS5 is the major aggrecanase in mouse cartilage *in vivo* and *in vitro*. *Nature.* 2005;434:648-52.
191. Cawston TE, Wilson AJ. Understanding the role of tissue degrading enzymes and their inhibitors in development and disease. *Best Pract Res Clin Rheumatol.* 2006;20:983-1002.
192. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res.* 2006;69:562-73.
193. Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci U S A.* 1990; 87:5578-82.
194. Cao J, Rehemtulla A, Pavlaki M, *et al*. Furin directly cleaves proMMP-2 in the trans-Golgi network resulting in a nonfunctioning proteinase. *J Biol Chem.* 2005;280:10974-80.
195. Lovejoy B, Cleasby A, Hassell AM, *et al*. Structure of the catalytic domain of fibroblast collagenase complexed with an inhibitor. *Science.* 1994;263:375-77.
196. Zhang Y, Dean WL, Gray RD. Cooperative binding of Ca2+ to human interstitial collagenase assessed by circular dichroism, fluorescence, and catalytic activity. *J Biol Chem.* 1997;272:1444-47.
197. Rosenblum G, Van den Steen PE, Cohen SR, *et al*. Insights into the structure and domain flexibility of full-length pro-matrix metalloproteinase-9/gelatinase B. *Structure.* 2007;15:1227-36.
198. Piccard H, Van den Steen PE, Opdenakker G. Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. *J Leukoc Biol.* 2007;81:870-892.
199. Toth M, Osenkowski P, Hesek D, *et al*. Cleavage at the stem region releases an active ectodomain of the membrane type 1 matrix metalloproteinase. *Biochem J.* 2005;387:497-506.

200. Toth M, Sohail A, Mobashery S, *et al*. MT1-MMP shedding involves an ADAM and is independent of its localization in lipid rafts. *Biochem Biophys Res Commun.* 2006;350:377-84.
201. Abraham M, Shapiro S, Lahat N, *et al*. The role of IL-18 and IL-12 in the modulation of matrix metalloproteinases and their tissue inhibitors in monocytic cells. *Int Immunol.* 2002;14:1449-57.
202. Fujisaki K, Tanabe N, Suzuki N, *et al*. The effect of IL-1alpha on the expression of matrix metalloproteinases, plasminogen activators, and their inhibitors in osteoblastic ROS 17/2.8 cells. *Life Sci.* 2006;78:1975-82.
203. Im HJ, Muddasani P, Natarajan V, *et al*. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes. *J Biol Chem.* 2007;282:11110-11121.
204. Johnatty RN, Taub DD, Reeder SP, *et al*. Cytokine and chemokine regulation of proMMP-9 and TIMP-1 production by human peripheral blood lymphocytes. *J Immunol.* 1997;158:2327-33.
205. Kelley MJ, Rose AY, Song K, *et al*. Synergism of TNF and IL-1 in the induction of matrix metalloproteinase-3 in trabecular meshwork. *Invest Ophthalmol Vis Sci.* 2007;48:2634-43.
206. Nelson KK, Ranganathan AC, Mansouri J, *et al*. Elevated sod2 activity augments matrix metalloproteinase expression: evidence for the involvement of endogenous hydrogen peroxide in regulating metastasis. *Clin Cancer Res.* 2003;9:424-32.
207. Robinson SC, Scott KA, Balkwill FR. Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNF-alpha. *Eur J Immunol.* 2002;32:404-12.
208. Rutter JL, Benbow U, Coon CI, *et al*. Cell-type specific regulation of human interstitial collagenase-1 gene expression by interleukin-1 beta (IL-1 beta) in human fibroblasts and BC-8701 breast cancer cells. *J Cell Biochem.* 1997;66:322-36.

209. Solis-Herruzo JA, Rippe RA, Schrum LW, *et al*. Interleukin-6 increases rat metalloproteinase-13 gene expression through stimulation of activator protein 1 transcription factor in cultured fibroblasts. *J Biol Chem.* 1999;274:30919-26.
210. Werle M, Schmal U, Hanna K, *et al*. MCP-1 induces activation of MAP-kinases ERK, JNK and p38 MAPK in human endothelial cells. *Cardiovasc Res.* 2002;56:284-92.
211. Xie S, Issa R, Sukkar MB, *et al*. Induction and regulation of matrix metalloproteinase-12 in human airway smooth muscle cells. *Respir Res.* 2005;6:148.
212. Liao EY, Luo XH. Effects of 17beta-estradiol on the expression of matrix metalloproteinase-1, -2 and tissue inhibitor of metalloproteinase-1 in human osteoblast-like cell cultures. *Endocrine.* 2001;15:291-95.
213. Ma Z, Chang MJ, Shah RC, *et al*. Interferon-gamma-activated STAT-1alpha suppresses MMP-9 gene transcription by sequestration of the coactivators CBP/p300. *J Leukoc Biol.* 2005;78:515-23.
214. Mackay AR, Ballin M, Pelina MD, *et al*. Effect of phorbol ester and cytokines on matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in tumor and normal cell lines. *Invasion Metastasis.* 1992;12:168-84.
215. Yuan W, Varga J. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. *J Biol Chem.* 2001;276:38502-10.
216. Gebbia JA, Coleman JL, Benach JL. Selective induction of matrix metalloproteinases by Borrelia burgdorferi via toll-like receptor 2 in monocytes. *J Infect Dis.* 2004;189:113-19.
217. Malik N, Greenfield BW, Wahl AF, *et al*. Activation of human monocytes through CD40 induces matrix metalloproteinases. *J Immunol.* 1996;156:3952-60.
218. Moore C, Shen XD, Gao F, *et al*. Fibronectin-alpha4beta1 integrin interactions regulate metalloproteinase-9 expression in steatotic liver ischemia and reperfusion injury. *Am J Pathol.* 2007;170:567-77.

219. Sudbeck BD, Pilcher BK, Welgus HG, *et al*. Induction and repression of collagenase-1 by keratinocytes is controlled by distinct components of different extracellular matrix compartments. *J Biol Chem.* 1997;272:22103-10.
220. Xie B, Laouar A, Huberman E. Fibronectin-mediated cell adhesion is required for induction of 92-kDa type IV collagenase/gelatinase (MMP-9) gene expression during macrophage differentiation. The signaling role of protein kinase C-beta. *J Biol Chem.* 1998;273:11576-82.
221. Vincenti MP, Brinckerhoff CE. Signal transduction and cell-type specific regulation of matrix metalloproteinase gene expression: can MMPs be good for you? *J Cell Physiol.* 2007;213:355-64.
222. Couillard J, Demers M, Lavoie G, *et al*. The role of DNA hypomethylation in the control of stromelysin gene expression. *Biochem Biophys Res Commun.* 2006;342:1233-39.
223. Martens JH, Verlaan M, Kalkhoven E, *et al*. Cascade of distinct histone modifications during collagenase gene activation. *Mol Cell Biol.* 2003;23:1808-16.
224. Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. *J Biol Chem.* 2001;276:7549-58.
225. Rutter JL, Mitchell TI, Buttice G, *et al*. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res.* 1998;58:5321-25.
226. Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J *Cell Physiol.* 2007;211:19-26.
227. Fahling M, Steege A, Perlewitz A, *et al*. Role of nucleolin in posttranscriptional control of MMP-9 expression. *Biochim Biophys Acta.* 2005;1731:32-40.
228. Chakrabarti S, Zee JM, Patel KD. Regulation of matrix metalloproteinase-9 (MMP-9) in TNF-stimulated neutrophils: novel pathways for tertiary granule release. *J Leukoc Biol.* 2006;79:214-22.
229. Kang T, Yi J, Guo A, *et al*. Subcellular distribution and cytokine- and chemokine-regulated secretion of leukolysin/MT6-MMP/MMP-25 in neutrophils. *J Biol Chem.* 2001;276:21960-21968.

230. Wiehler S, Cuvelier SL, Chakrabarti S, *et al*. p38 MAP kinase regulates rapid matrix metalloproteinase-9 release from eosinophils. *Biochem Biophys Res Commun.* 2004;315:463-70.
231. Zucker S, Hymowitz M, Conner CE, *et al*. Rapid trafficking of membrane type 1-matrix metalloproteinase to the cell surface regulates progelatinase a activation. *Lab Invest.* 2002;82:1673-84.
232. Rahat MA, Marom B, Bitterman H, *et al*. Hypoxia reduces the output of matrix metalloproteinase-9 (MMP-9) in monocytes by inhibiting its secretion and elevating membranal association. *J Leukoc Biol.* 2006;79:706-18.
233. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003;92:827-39.
234. Galazka G, Windsor LJ, Birkedal-Hansen H, *et al*. APMA (4-aminophenylmercuric acetate) activation of stromelysin-1 involves protein interactions in addition to those with cysteine-75 in the propeptide. *Biochemistry.* 1996;35:11221-27.
235. Suzuki K, Enghild JJ, Morodomi T, *et al*. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry.* 1990;29:10261-70.
236. Bannikov GA, Karelina TV, Collier IE, *et al*. Substrate binding of gelatinase B induces its enzymatic activity in the presence of intact propeptide. *J Biol Chem.* 2002;277:16022-27.
237. Rosenblum G, Meroueh S, Toth M, *et al*. Molecular Structures and Dynamics of the Stepwise Activation Mechanism of a Matrix Metalloproteinase Zymogen: Challenging the Cysteine Switch Dogma. *J Am Chem Soc.* 2007;129:13566-74.
238. Fu X, Parks WC, Heinecke JW. Activation and silencing of matrix metalloproteinases. *Semin Cell Dev Biol.* 2007.
239. Fernandez CA, Yan L, Louis G, *et al*. The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients. *Clin Cancer Res.* 2005;11:5390-5395.

240. Kubben FJ, Sier CF, Hawinkels LJ, *et al*. Clinical evidence for a protective role of lipocalin-2 against MMP-9 autodegradation and the impact for gastric cancer. *Eur J Cancer.* 2007;43:1869-76.
241. Van den Steen PE, Opdenakker G, Wormald MR, *et al*. Matrix remodelling enzymes, the protease cascade and glycosylation. *Biochim Biophys Acta.* 2001;1528:61-73.
242. Jiang A, Lehti K, Wang X, *et al*. Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. *Proc Natl Acad Sci U S A.* 2001;98:13693-98.
243. Lafleur MA, Mercuri FA, Ruangpanit N, *et al*. Type I collagen abrogates the clathrin-mediated internalization of membrane type 1 matrix metalloproteinase (MT1-MMP) via the MT1-MMP hemopexin domain. *J Biol Chem.* 2006;281:6826-40.
244. McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol.* 2001;13:534-40.
245. Sawicki G, Leon H, Sawicka J, *et al*. Degradation of myosin light chain in isolated rat hearts subjected to ischemia-reperfusion injury: a new intracellular target for matrix metalloproteinase-2. *Circulation.* 2005;112:544-52.
246. Wang W, Schulze CJ, Suarez-Pinzon WL, *et al*. Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. *Circulation.* 2002;106:1543-49.
247. D'Alessio S, Ferrari G, Cinnante K, *et al*. Tissue inhibitor of metalloproteinases-2 binding to membrane-type 1 matrix metalloproteinase induces MAPK activation and cell growth by a non-proteolytic mechanism. *J Biol Chem.* 2008;283:87-99.
248. Lambert E, Dasse E, Haye B, *et al*. TIMPs as multifacial proteins. *Crit Rev Oncol Hematol.* 2004;49:187-98.
249. Eickelberg O, Kohler E, Reichenberger F, *et al*. Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-beta1 and TGF-beta3. *Am J Physiol.* 1999;276:L814-L824.

250. Cameron EE, Bachman KE, Myohanen S, *et al*. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet.* 1999;21:103-7.
251. Anderson CL, Brown CJ. Polymorphic X-chromosome inactivation of the human TIMP1 gene. *Am J Hum Genet.* 1999;65:699-708.
252. Anderson CL, Brown CJ. Variability of X chromosome inactivation: effect on levels of TIMP1 RNA and role of DNA methylation. *Hum Genet.* 2002;110:271-78.
253. Ivanova T, Vinokurova S, Petrenko A, *et al*. Frequent hypermethylation of 5' flanking region of TIMP-2 gene in cervical cancer. *Int J Cancer.* 2004;108:882-86.
254. Chirco R, Liu XW, Jung KK, *et al*. Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev.* 2006;25:99-113.
255. Langton KP, McKie N, Curtis A, *et al*. A novel tissue inhibitor of metalloproteinases-3 mutation reveals a common molecular phenotype in Sorsby's fundus dystrophy. *J Biol Chem.* 2000;275:27027-31.
256. Baugh MD, Perry MJ, Hollander AP, *et al*. Matrix metalloproteinase levels are elevated in inflammatory bowel disease. *Gastroenterology.* 1999;117:814-22.
257. Bister VO, Salmela MT, Karjalainen-Lindsberg ML, *et al*. Differential expression of three matrix metalloproteinases, MMP-19, MMP-26, and MMP-28, in normal and inflamed intestine and colon cancer. *Dig Dis Sci*. 2004;49:653-61.
258. Heuschkel RB, MacDonald TT, Monteleone G, *et al*. Imbalance of stromelysin-1 and TIMP-1 in the mucosal lesions of children with inflammatory bowel disease. *Gut.* 2000;47:57-62.
259. Saarialho-Kere UK, Vaalamo M, Puolakkainen P, *et al*. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. *Am J Pathol.* 1996;148:519-26.
260. Stallmach A, Chan CC, Ecker KW, *et al*. Comparable expression of matrix metalloproteinases 1 and 2 in pouchitis and ulcerative colitis. *Gut.* 2000;47:415-22.

261. Vaalamo M, Karjalainen-Lindsberg ML, Puolakkainen P, *et al*. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. *Am J Pathol.* 1998;152:1005-14.
262. von Lampe B, Barthel B, Coupland SE, *et al*. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. *Gut.* 2000;47:63-73.
263. Wheatcroft AC, Hollander AP, Croucher LJ, *et al*. Evidence of *in situ* stability of the type IV collagen triple helix in human inflammatory bowel disease using a denaturation specific epitope antibody. *Matrix Biol.* 1999;18:361-72.
264. Corbel M, Caulet-Maugendre S, Germain N, *et al*. Inhibition of bleomycin-induced pulmonary fibrosis in mice by the matrix metalloproteinase inhibitor batimastat. *J Pathol.* 2001;193:538-45.
265. Tan RJ, Fattman CL, Niehouse LM, *et al*. Matrix metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. *Am J Respir Cell Mol Biol.* 2006;35:289-97.
266. Warnaar N, Hofker HS, Maathuis MH, *et al*. Matrix metalloproteinases as profibrotic factors in terminal ileum in Crohn's disease. *Inflamm Bowel Dis.* 2006;12:863-69.
267. McKaig BC, McWilliams D, Watson SA, *et al*. Expression and regulation of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinases by intestinal myofibroblasts in inflammatory bowel disease. *Am J Pathol.* 2003;162:1355-60.
268. Roderfeld M, Weiskirchen R, Wagner S, *et al*. Inhibition of hepatic fibrogenesis by matrix metalloproteinase-9 mutants in mice. *FASEB J.* 2006;20:444-54.
269. Opdenakker G, Dillen C, Fiten P, *et al*. Remnant epitopes, autoimmunity and glycosylation. *Biochim Biophys Acta.* 2006;1760:610-615.
270. Castaneda FE, Walia B, Vijay-Kumar M, *et al*. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. *Gastroenterology.* 2005;129:1991-2008.

271. Kobayashi K, Arimura Y, Goto A, *et al*. Therapeutic implications of the specific inhibition of causative matrix metalloproteinases in experimental colitis induced by dextran sulphate sodium. *J Pathol.* 2006;209:376-83.
272. Medina C, Videla S, Radomski A, *et al*. Therapeutic effect of phenantroline in two rat models of inflammatory bowel disease. *Scand J Gastroenterol.* 2001;36:1314-19.
273. Garg P, Rojas M, Ravi A, *et al*. Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis: contrasting role of gelatinases in the pathogenesis of colitis. *J Immunol.* 2006;177:4103-12.
274. Van den Steen PE, Proost P, Wuyts A, *et al*. Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-alpha and leaves RANTES and MCP-2 intact. *Blood.* 2000;96:2673-81.
275. Anderson F, Game BA, Atchley D, *et al*. IFN-gamma pretreatment augments immune complex-induced matrix metalloproteinase-1 expression in U937 histiocytes. *Clin Immunol.* 2002;102:200-207.
276. Dumin JA, Dickeson SK, Stricker TP, *et al*. Pro-collagenase-1 (matrix metalloproteinase-1) binds the alpha(2)beta(1) integrin upon release from keratinocytes migrating on type I collagen. *J Biol Chem.* 2001;276:29368-74.
277. Ghaffari A, Li Y, Karami A, *et al*. Fibroblast extracellular matrix gene expression in response to keratinocyte-releasable stratifin. *J Cell Biochem.* 2006;98:383-93.
278. Abe M, Kawamoto K, Okamoto H, *et al*. Induction of collagenase-2 (matrix metalloproteinase-8) gene expression by interleukin-1beta in human gingival fibroblasts. *J Periodontal Res.* 2001;36:153-59.
279. Devarajan P, Mookhtiar K, Van Wart H, *et al*. Structure and expression of the cDNA encoding human neutrophil collagenase. *Blood.* 1991;77:2731-38.
280. Im HJ, Li X, Muddasani P, *et al*. Basic fibroblast growth factor accelerates matrix degradation via a neuro-endocrine pathway in human adult articular chondrocytes. *J Cell Physiol.* 2008;215:452-63.

281. Yang CM, Chien CS, Yao CC, *et al*. Mechanical strain induces collagenase-3 (MMP-13) expression in MC3T3-E1 osteoblastic cells. *J Biol Chem.* 2004;279:22158-65.
282. Galboiz Y, Shapiro S, Lahat N, *et al*. Modulation of monocytes matrix metalloproteinase-2, MT1-MMP and TIMP-2 by interferon-gamma and -beta: implications to multiple sclerosis. *J Neuroimmunol.* 2002;131:191-200.
283. Haque NS, Fallon JT, Pan JJ, *et al*. Chemokine receptor-8 (CCR8) mediates human vascular smooth muscle cell chemotaxis and metalloproteinase-2 secretion. *Blood.* 2004;103:1296-304.
284. Wisithphrom K, Windsor LJ. The effects of tumor necrosis factor-alpha, interleukin-1beta, interleukin-6, and transforming growth factor-beta1 on pulp fibroblast mediated collagen degradation. *J Endod.* 2006;32:853-61.
285. Yakubenko VP, Lobb RR, Plow EF, *et al*. Differential induction of gelatinase B (MMP-9) and gelatinase A (MMP-2) in T lymphocytes upon alpha(4)beta(1)-mediated adhesion to VCAM-1 and the CS-1 peptide of fibronectin. *Exp Cell Res.* 2000;260:73-84.
286. Brown RD, Jones GM, Laird RE, *et al*. Cytokines regulate matrix metalloproteinases and migration in cardiac fibroblasts. *Biochem Biophys Res Commun.* 2007;362:200-205.
287. Ichiyama T, Kajimoto M, Hasegawa M, *et al*. Cysteinyl leukotrienes enhance tumour necrosis factor-alpha-induced matrix metalloproteinase-9 in human monocytes/macrophages. *Clin Exp Allergy.* 2007;37:608-14.
288. Owen CA, Hu Z, Barrick B, *et al*. Inducible expression of tissue inhibitor of metalloproteinases-resistant matrix metalloproteinase-9 on the cell surface of neutrophils. *Am J Respir Cell Mol Biol.* 2003;29:283-94.
289. Blindt R, Vogt F, Lamby D, *et al*. Characterization of differential gene expression in quiescent and invasive human arterial smooth muscle cells. *J Vasc Res.* 2002;39:340-352.
290. Hanemaaijer R, Koolwijk P, le Clercq L, *et al*. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. *Biochem J.* 1993;296 (Pt 3):803-9.

291. Mix KS, Attur MG, Al Mussawir H, *et al*. Transcriptional repression of matrix metalloproteinase gene expression by the orphan nuclear receptor NURR1 in cartilage. *J Biol Chem.* 2007;282:9492-504.
292. Tamai K, Ishikawa H, Mauviel A, *et al*. Interferon-gamma coordinately upregulates matrix metalloprotease (MMP)-1 and MMP-3, but not tissue inhibitor of metalloproteases (TIMP), expression in cultured keratinocytes. *J Invest Dermatol.* 1995;104:384-90.
293. Unoki H, Bujo H, Jiang M, *et al*. Macrophages regulate tumor necrosis factor-alpha expression in adipocytes through the secretion of matrix metalloproteinase-3. *Int J Obes (Lond).* 2008;32:902-11.
294. Warstat K, Pap T, Klein G, *et al*. Co-activation of synovial fibroblasts by laminin-111 and transforming growth factor-beta induces expression of matrix metalloproteinases 3 and 10 independently of nuclear factor-kappaB. *Ann Rheum Dis.* 2008;67:559-62.
295. Bar-Or A, Nuttall RK, Duddy M, *et al*. Analyses of all matrix metalloproteinase members in leukocytes emphasize monocytes as major inflammatory mediators in multiple sclerosis. *Brain.* 2003;126:2738-49.
296. Rechardt O, Elomaa O, Vaalamo M, *et al*. Stromelysin-2 is upregulated during normal wound repair and is induced by cytokines. *J Invest Dermatol.* 2000;115:778-87.
297. Bertram C, Hass R. MMP-7 is involved in the aging of primary human mammary epithelial cells (HMEC). *Exp Gerontol.* 2008;43:209-17.
298. Graesslin O, Cortez A, Fauvet R, *et al*. Metalloproteinase-2, -7 and -9 and tissue inhibitor of metalloproteinase-1 and -2 expression in normal, hyperplastic and neoplastic endometrium: a clinical-pathological correlation study. *Ann Oncol.* 2006;17:637-45.
299. Pirila E, Korpi JT, Korkiamaki T, *et al*. Collagenase-2 (MMP-8) and matrilysin-2 (MMP-26) expression in human wounds of different etiologies. *Wound Repair Regen.* 2007;15:47-57.
300. Ripley D, Tunuguntla R, Susi L, *et al*. Expression of matrix metalloproteinase-26 and tissue inhibitors of metalloproteinase-3 and -4 in normal ovary and ovarian carcinoma. *Int J Gynecol Cancer.* 2006;16:1794-800.

301. Uria JA, Lopez-Otin C. Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the minimal domain organization required for secretion, latency, and activity. *Cancer Res.* 2000;60:4745-51.
302. Cury PR, Canavez F, de Araujo VC, *et al*. Substance P regulates the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinase in cultured human gingival fibroblasts. *J Periodontal Res.* 2008;43:255-60.
303. Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol.* 2000;279:G245-G249.
304. Lambert CA, Colige AC, Munaut C, *et al*. Distinct pathways in the over-expression of matrix metalloproteinases in human fibroblasts by relaxation of mechanical tension. *Matrix Biol.* 2001;20:397-408.
305. Schonherr E, Schaefer L, O'Connell BC, *et al*. Matrix metalloproteinase expression by endothelial cells in collagen lattices changes during co-culture with fibroblasts and upon induction of decorin expression. *J Cell Physiol.* 2001;187:37-47.
306. Toft-Hansen H, Nuttall RK, Edwards DR, *et al*. Key metalloproteinases are expressed by specific cell types in experimental autoimmune encephalomyelitis. *J Immunol.* 2004;173:5209-18.
307. Milner JM, Rowan AD, Cawston TE, *et al*. Metalloproteinase and inhibitor expression profiling of resorbing cartilage reveals pro-collagenase activation as a critical step for collagenolysis. *Arthritis Res Ther.* 2006;8:R142.
308. Nocito A, Dahm F, Jochum W, *et al*. Serotonin regulates macrophage-mediated angiogenesis in a mouse model of colon cancer allografts. *Cancer Res.* 2008;68:5152-58.
309. Beck IM, Muller M, Mentlein R, *et al*. Matrix metalloproteinase-19 expression in keratinocytes is repressed by transcription factors Tst-1 and Skn-1a: implications for keratinocyte differentiation. *J Invest Dermatol.* 2007;127:1107-14.
310. van Horssen J, Vos CM, Admiraal L, *et al*. Matrix metalloproteinase-19 is highly expressed in active multiple sclerosis lesions. *Neuropathol Appl Neurobiol.* 2006;32:585-93.

311. Zhang Y, Yan Q, Li W, *et al*. Fluoride down-regulates the expression of matrix metalloproteinase-20 in human fetal tooth ameloblast-lineage cells *in vitro*. *Eur J Oral Sci.* 2006;114 Suppl 1:105-10.
312. Skoog T, Elomaa O, Pasonen-Seppanen SM, *et al*. Matrix Metalloproteinase-21 Expression Is Associated with Keratinocyte Differentiation and Upregulated by Retinoic Acid in HaCaT Cells. *J Invest Dermatol.* 2008.
313. Velasco G, Pendas AM, Fueyo A, *et al*. Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members. *J Biol Chem.* 1999;274:4570-4576.
314. Saarialho-Kere U, Kerkela E, Jahkola T, *et al*. Epilysin (MMP-28) expression is associated with cell proliferation during epithelial repair. *J Invest Dermatol.* 2002;119:14-21.