Available online at www.sciencedirect.com
SCIENCE DIRECT
Gene 356 (2005) 193 – 202
GENE
SECTION
FUNCTIONAL GENOMICS
www.elsevier.com/locate/gene

The proximal promoter of the human *cathepsin G* gene conferring myeloid-specific expression includes C/EBP, *c*-myb and PU.1 binding sites

Andreas Lennartsson, Daniel Garwicz, Anders Lindmark, Urban Gullberg*

Division of Hematology and Transfusion Medicine, Lund University, BMC, C14, S-221 84 Lund, Sweden

Received 18 November 2004; received in revised form 18 April 2005; accepted 4 May 2005
Available online 12 July 2005
Received by D. Higgs

Abstract

Cathepsin G is a hematopoietic serine protease stored in the azurophil granules of neutrophil granulocytes. The mRNA of cathepsin G is transiently expressed during the promyelocyte stage of neutrophil maturation. The protease plays several roles in inflammatory actions of neutrophils, such as bactericidal effects. A human *cathepsin G* gene fragment of 6 kb directs a promyelocyte-specific expression in transgenic mice, indicating the presence of necessary *cis*-acting elements. However, neither the precise architecture of the promoter, nor the *trans*-acting factors responsible for its activation, have been characterized. In the present work, 2.6 kb upstream of the translation start site of the human *cathepsin G* gene was cloned. When transfected to monoblast-like U937 or to acute promyelocytic leukemia NB4 cells, both expressing endogenous cathepsin G, the initial 360 bp upstream of the translation start were sufficient to direct a strong expression of a luciferase reporter gene. No expression was observed in erythroid K562 control cells. Further deletions revealed three major regulatory regions containing the consensus binding-sites for the transcription factors C/EBP, *c*-myb and PU.1. Moreover, a GC-rich region, similar to a *cis*-element in the *proteinase 3* promoter, was identified. Direct binding of the *trans*-factors C/EBPα, C/EBPε, *c*-myb and PU.1 to the promoter was shown by chromatin immunoprecipitation. The functional significance of the *cis*-elements was verified by site-directed mutagenesis. Mutations of the putative PU.1 site moderately decreased the activity of the promoter in monoblastic U937 cells, but not in promyelocytic NB4 cells. Separate mutations of the putative C/EBP binding site, *c*-myb-binding site or the GC-rich element resulted in a dramatically reduced transcriptional activity in both cell lines, suggesting cooperation between corresponding *trans*-factors.

Keywords: Serine proteases; Granulopoiesis; Azurophil; Neutrophil; Transcriptional regulation

1. Introduction

The hematopoietic serine proteases constitute a superfamily of enzymes stored in cytoplasmic granules of hematopoietic cells, showing capacity for regulated secretion. Cathepsin G is one of four serine protease family members stored in the azurophil granules of neutrophil granulocytes; the neutrophil serine protease family also includes leukocyte elastase, proteinase 3 and the enzymatically inactive protease homologue azurocidin (Gullberg et al., 1997).

Cathepsin G has been proposed to play several roles in inflammation and host defence. Purified cathepsin G is cytotoxic against, or inhibits the growth of, a number of microbes (Shafer and Onunka, 1989; Shafer et al., 1991; Guyonnet et al., 1991). Elimination of cathepsin G in transgenic mice results in no obvious defects in microbial clearance, but combined deletion of the *cathepsin G* and

Abbreviations: bp, base pair; TNF, tumor necrosis factor; IL, interleukin; DA, Dalton; FCS, fetal calf serum; PCR, polymerase chain reaction; ChIP, chromatin immunoprecipitation; BPI, bactericidal/permeability increasing protein; SEM, standard error of the mean; SDS-PAGE, sodium doceyl sulfate-polyacrylamide gel electrophoresis.

* Corresponding author. Tel.: +46 46 222 07 29; fax: +46 46 18 44 93.
E-mail address: urban.gullberg@med.lu.se (U. Gullberg).

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.05.004

leukocyte *elastase* genes leads to increased susceptibility to bacterial challenges (Tkalcevic et al., 2000). Besides localization in azurophil granules, cathepsin G is present on the cell surface of neutrophils after degranulation, possibly enhancing chemotactic signaling (Maison et al., 1991; Tkalcevic et al., 2000; Lomas et al., 1995). Cleavage of several blood clotting factors (e.g. coagulation factor V) and the thrombin receptor, leading to functional activation, are examples of potential extracellular roles of cathepsin G (Allen and Tracy, 1995; Turkington et al., 1986). Uncontrolled proteolytic action of cathepsin G may contribute to tissue destruction seen in various chronic inflammatory disorders, such as allergic rhinitis (Westin et al., 1999). It has also been proposed that cathepsin G plays a role in tissue remodeling at sites of wounding or tissue injury, by cleavage and inactivation of chemoattractants for neutrophils, such as TNF-α (Scuderi et al., 1991), IL-1 (Hazuda et al., 1990) and IL-8 (Padrines et al., 1994). Consistently, cathepsin G deficient mice have an increased amount of neutrophils at inflammatory sites, which may be due to a high concentration of chemoattractants in the wound fluid (Abbott et al., 1998).

Neutrophil serine proteases are mainly synthesized during the promyelocytic stage of myeloid differentiation, and after processing into mature enzymes the proteases are stored in azurophil granules as proteolytically active enzymes (Gullberg et al., 1999; Cowland and Borregaard, 1999). No sorting motifs for sorting to granules have yet been identified (Gullberg et al., 1999). Rather, it has been proposed that differentiation-related transcriptional control of the expression of the *cathepsin G* gene and other granule components results in sorting into distinct granule subtypes (Arnljots et al., 1998). Identification of important transactivating factors for the promyelocyte specific expression of *elastase* and *proteinase 3* have been reported (Lutz et al., 2001; Nuchprayoon et al., 1997; Oelgeschlager et al., 1996; Sturrock et al., 1996, 2004). These factors include the trancriptional activators PU.1, *c*-myb, C/EBP and a 40 kDa protein binding to a GC-rich *cis*-element. In contrast to the *elastase* and *proteinase 3* genes, located on chromosome 19, the *cathepsin G* gene is located on chromosome 14 in a cluster with the genes encoding the cytotoxic T-cell protease *granzyme B* and *granzyme H* (Hanson et al., 1990; Yousef et al., 2003). Moreover, *cathepsin G* seems be expressed somewhat later than *elastase* and *proteinase 3* (Garwicz et al., 2005). The tissue- and maturation-specific expression of *cathepsin G* has been linked to a region stretching from 2.5 kb upstream to 0.8 kbp downstream of the first and last exon, respectively, as judged by its capacity to dictate expression in promyelocytes of transgenic mice (Grisolano et al., 1994). Detailed molecular analysis of the promoter of *cathepsin G* including transactivating factors is, however, not available. Given the distinct chromosomal localization of *cathepsin G*, as compared to the *proteinase 3* and leukocyte *elastase* genes, we asked whether the proximal promoter shows a similar functional and structural organization. Our results show that within 360 bp upstream of the translation start a proximal promoter is contained, including binding sites for PU.1, *c*-myb, C/EBP, and a GC-rich element, similar to the promoters of *proteinase 3* and *leukocyte elastase*. Moreover, the C/EBP binding site, *c*-myb binding sites and the GC-rich element show pronounced functional synergy, conferring strong expression from the promoter in myeloid cells.

2. Materials and methods

2.1. Cell culture

Monoblastic U937, promyelocytic NB4 and erythroid K562 were maintained in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, GIBCO). Exponentially growing cells were used for all experiments.

2.2. Genomic cloning

Cloning of human genomic DNA upstream of the transcription start of the *cathepsin G* gene was performed by PCR, utilizing the human Genomewalker™-kit (Clontech), according to the manufacturer's recommendations. From published cDNA sequence (Hohn et al., 1989) oligonucleotide downstream primers specific for *cathepsin G* were synthesized for use in nested PCR: adapt primer 5′-AGGAGAAAGGCCAGCAGAAGC-3′ and nested primer 5′-TCAACT*GCTAGC*CTTTCCTGAAAGGCTGCC-CAGTC-3′ (NheI restriction site underlined). Both primers bind to the published cDNA sequence of *cathepsin G*. All PCRs were performed in a DNA thermal cycler 480 (Perkin Elmer). The resulting PCR products 1.2 kbp and 2.6 kbp, respectively, were cloned into luciferase reporter vector pGL3/basic (Promega) using MluI and NheI restriction sites, thus creating the corresponding vectors pGL3/1.2 and pGL3/2.6. Sequencing (Cybergene AB, Huddinge, Sweden) was performed to control the identity, compared with the previously published 5′ flanking region of the *cathepsin G* gene [GeneBank, M59717.1]. As previously reported, a potential TATA-box and CAAT-box at position −57 and −97, respectively, are present in the putative promoter region (numeration from the translation start site) (Hohn et al., 1989).

2.3. Deletion and mutation constructs

To create sequential deletions of the promoter region, the nested primer described above was used as downstream primer, together with upstream primers complementary to sequences in the promoter at 807 bp, 564 bp, 360 bp, 300 bp, 250 bp, 200 bp and 150 bp, respectively. The upstream primers included a restriction site for Mlu I. For site directed mutagenesis of potential transcription factor binding sites, oligonucleotide primers including the desired mutations