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Research paper

Proteomic approach to the identification of novel delta-lactoferrin target genes: Characterization of DcpS, an mRNA scavenger decapping enzyme

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Abstract

The expression of the transcription factor ΔLf is deregulated in cancer cells. Its overexpression provokes cell cycle arrest along with antiproliferative effects and we recently showed that the *Skp1* gene promoter was a target of ΔLf. Skp1 belongs to the Skp1/Cullin-1/F-box ubiquitin ligase complex responsible for the ubiquitination and the proteosomal degradation of numerous cellular regulators. The transcriptional activity of ΔLf is highly controlled and negatively regulated by *O*-GlcNAc, a dynamic post-translational modification known to regulate the functions of many intracellular proteins. We, therefore, constructed a ΔLf-M4 mutant corresponding to a constitutively active ΔLf isoform in which all the glycosylation sites were mutated. In order to discover novel targets of ΔLf transcriptional activity and to investigate the impact of the *O*-GlcNAc regulation on this activity *in situ* we compared the proteome profiles of ΔLf- and ΔLf-M4-expressing HEK293 cells versus null plasmid transfected cells. A total of 14 differentially expressed proteins were visualized by 2D electrophoresis and silver staining and eight proteins were identified by mass spectrometry analyses (MALDI-TOF; LC-MS/MS), all of which were upregulated. The identified proteins are involved in several processes such as mRNA maturation and stability, cell viability, proteasomal degradation, protein and mRNA quality control. Among these proteins, only DcpS and TCPB were also upregulated at the mRNA level. Analysis of their respective promoters led to the detection of a *cis*-regulating element in the *DcpS* promoter. The $S1^{DcpS}$ is 80% identical to the S1 sequence previously described by He and Furmanski [Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA, Nature 373 (1995) 721–724]. Reporter gene analyses and ChIP assays demonstrated that ΔLf interacts specifically with the *DcpS* promoter *in vivo*. These data established that DcpS, a key enzyme in mRNA decay, is a new target of ΔLf transcriptional activity.

Keywords: Delta-lactoferrin; Transcription factor; Scavenger decapping enzyme DcpS; mRNA turnover; *O*-GlcNAc/P interplay; Proteomics

1. Introduction

The process of mRNA turnover is a critical mechanism for the regulation of gene expression, quality control of mRNA biogenesis and antiviral defenses. The major mRNA degradation pathways involve shortening the poly(A) tail, exonucleolytic decay and decapping (reviewed in refs. [1,2]). Among the decapping enzymes, DcpS, also known as the scavenger decapping enzyme, is involved in cap nucleotide metabolism [3,4]. Here, we show that delta-lactoferrin (ΔLf), a transcription factor involved in the regulation of cell cycle progression at the G1/S transition, enhances *DcpS* transcription.

First discovered as a transcript in normal tissues [5], ΔLf was found to be downregulated in cancer cells and in breast cancer biopsies [6]. Its expression level was of good prognosis value in human breast cancer with high concentrations

Abbreviations: ΔLf, delta-lactoferrin; DcpS, scavenger decapping enzyme; hDcp2, human decapping enzyme 2; Skp1, S-phase kinase 1; RPLP0, ribosomal protein, large, P0; ΔLfRE, ΔLf response element; NLS, nuclear localization signal; *O*-GlcNAc/P, *O*-glycosylation/phosphorylation.

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being associated with longer relapse-free and overall survival [6]. ΔLf is a lactoferrin isoform, the transcription of which starts at the alternative promoter P2 present in the first intron of the Lf gene [7]. The comparison of the two enhancer/promoter regions revealed that this gene is differentially transactivated [5,7]. The deregulation of Lf gene expression that occurs in tumors is mainly due to genetic and epigenetic changes [8–11]. The alternative selection of promoters produces an alternative N-terminal domain. Thus, compared to Lf, ΔLf is a protein devoid of the 45 first amino acid residues including the leader sequence, implying that it is a 73 kDa cytoplasmic isoform [12]. However, ΔLf was also observed in the nucleus [12,13] and a short bipartite nuclear localization signal at the C-terminus conserved in Lfs from different species, has been identified [14]. Recently, we showed that ΔLf is an efficient transcription factor interacting *in vivo* with a ΔLf response element (ΔLfRE) found in the *Skp1* promoter [14]. This specific GGCACTTGC sequence had previously been described [15] and found to be responsible for IL-1β transactivation by Lf [16]. Studies of the three-dimensional (3D) Lf structure indicated two putative DNA-binding domains (DBDs) located either at the N-terminus (residues 27–30 in Lf and 2–5 in ΔLf) and/or at the interlobe region [17,18].

ΔLf expression provokes antiproliferative effects, cell cycle arrest in S phase [12] and Skp1 upregulation [14]. At the G1-S transition, Skp1 (S phase kinase associated protein) belongs to the SCF (Skp1/Cullin-1/F-box ubiquitin ligase) complex responsible for the ubiquitination of cellular regulators such as cyclins and cyclin-dependent kinase (CDK) inhibitors leading to their degradation by the proteasome [19,20]. At the G2/M transition Skp1 belongs to the CBF3 complex involved in the preservation of genetic stability [21,22]. By upregulating Skp1 gene expression, ΔLf may survey cell cycle progression *via* the control of the proteasomal degradation of S phase actors. Thus, ΔLf transcriptional activity should be strongly controlled. The presence of putative *O*-*N*-acetylglucosaminylation (*O*-GlcNAc) sites (YinOYang 1.2 server, http://www.cbs.dtu.dk/services/YinOYang/) could imply a control of the transcriptional activity or the half-life of ΔLf *via* the balance between *O*-GlcNAc and phosphorylation as already described for other factors [23]. Four *O*-GlcNAc sites are present (Ser10, Ser227, Ser472 and Thr559) the mutation of which produces the constitutively active ΔLf-M4 mutant with a 2.5-fold increased transcriptional activity compared to wild type.

In order to identify factors that are differentially expressed in response to ΔLf or the ΔLf-M4 mutant isoform, we have undertaken a differential proteomic approach using 2D gel electrophoresis combined with mass spectrometry. Among the eight differentially expressed proteins described here, we identified DcpS as a new ΔLf target gene. DcpS is a member of the HIT family of pyrophosphatases which performs catalysis of the 5′ cap structure [24]. This cap, that has to be removed during mRNA decay, is involved in a variety of functions such as pre-mRNA splicing, export, stability and efficient translation [25–27]. Eukaryotic mRNA degradation proceeds through two main pathways. In the 3′–5′ mRNA decay pathway, degradation generates free m7GpppN that is hydrolyzed by DcpS, the scavenger decapping enzyme, generating m7GMP. In the 5′–3′ pathway, the cleavage of the cap of deadenylated mRNAs is performed by the hDcp2 decapping enzyme producing 5′-phosphorylated mRNA and m7GDP which is then converted to m7GMP by DcpS [3,24,28–30].

Our findings showed that ΔLf and its constitutively active mutant modulate the expression of proteins involved in the cell cycle, cell survival and mRNA turnover. Among them, DcpS was shown to be a new target of ΔLf transcriptional activity.

2. Materials and methods

2.1. Cell culture

Human HEK 293 cells (ATC CRL-1573) were kindly provided by Dr. J.-C. Dhalluin (INSERM U 524, Lille, France). Human cervical cancer HeLa cells (ATCC CCL-2) were a kind gift from Dr. T. Lefebvre (UGSF, UM5 8576 CNRS, Villeneuve d'Ascq, France). The Breast Cancer MDA-MB231 (ATCC HTB-26) cell line was kindly provided by M. Mareel (Laboratory of Experimental Cancerology, University Hospital, Ghent, Belgium). Cells were routinely grown in monolayers as previously described [12,14]. MDA-MB-231 cells stably transfected with ΔLf (MDA-MB-231-ΔLf) were produced as in [12,14]. Expression of ΔLf is induced by doxycycline (2 μg/ml) [12] in these ΔLf-expressing cell lines. Cell culture materials were obtained from Dutscher (France), and culture media and additives from Cambrex Corporation (NJ, USA) and Invitrogen (UK).

2.2. DNA and RNA isolation

Genomic DNA was extracted from HEK 293 cells as previously described [33] and purified using QIAprep Spin Miniprep Kit (Qiagen, Germany). Total RNA was extracted from cell cultures using the RNeasy Mini Kit (Qiagen) according to the manufacturer's specifications. The purity of the extracts was checked by measuring the ratio of the absorbance at 260 nm and 280 nm using a nanodrop ND-1000 spectrophotometer (Labtech International, UK) and their integrity was visualized on a BET-agarose gel.

2.3. Transfection

Transfections were performed using the Dreamfect® reagent (OZ Biosciences, France), according to the manufacturer's instructions. After incubation for 24 h, cells were washed with NaCl/Pi. They were then lysed in appropriate buffer, either for total RNA preparation or for protein extracts. Protease inhibitor (Pefablock SC and Complete, Roche, Switzerland) was added to protein extracts. Except for transfections dedicated to the proteomic analysis, each transfection