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ORIGINAL ARTICLE

Chromosome region maintenance-1 (CRM1) regulates apoptosis of intestinal epithelial cells via p27kip1 in Crohn's disease

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Summary
Objective: To investigate the role of chromosome region maintenance-1 (CRM1) in Crohn's disease (CD) and its potential pathological mechanisms.
Methods: The expression and distribution of CRM1 in mucosal biopsies from patients with active CD and normal controls were detected by immunohistochemistry (IHC). We established a murine model of acute colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS). Western blot was performed to investigate the expression levels of CRM1, apoptotic markers (active caspase-3 and cleaved PARP), p27kip1 and p-p27ser10. IHC was performed to evaluate the distribution of CRM1, and double immunofluorescence (IF) was performed to evaluate the co-localization of CRM1 and active capase-3. Cells of the human intestinal epithelial cell line HT-29 were incubated with tumor necrosis factor-α (TNF-α) to establish an apoptotic in vitro model. Western blot was performed to determine the expression levels of CRM1, active caspase-3, cleaved PARP and p-p27ser10. Cytoplasmic and nuclear extracts were assessed to examine the translocation of CRM1. The interaction between CRM1 and p27kip1 was assessed by co-immunoprecipitation (co-IP) assays. Furthermore, we used small interfering RNA (siRNA) to knock down the protein

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expression of CRM1 in HT-29 cells and then measured the expression of active caspase-3, cleaved PARP and p-p27ser10. Flow cytometry was used to determine the effect of CRM1 on intestinal epithelial cell (IEC) apoptosis.

Results: We observed up-regulation of CRM1 accompanied by elevated levels of IEC apoptotic markers (active caspase-3 and cleaved PARP) and p-p27ser10 in IECs of patients with active CD and in TNBS-induced colitis model cells. However, the expression of p27kip1 was negatively correlated with the expression patterns of CRM1, p-p27ser10 and apoptotic biochemical markers. Co-localization of CRM1 and active caspase-3 in IECs of the TNBS group further indicated the possible involvement of CRM1 in IEC apoptosis. By employing TNF-α-treated HT-29 cells as an in vitro IEC apoptosis model, we found that the expression levels of CRM1 and p-p27ser10 were in accordance with active caspase-3 and cleaved PARP. In addition, immunoprecipitation confirmed the physical interaction between CRM1 and p27kip1. siRNA knockdown of CRM1 significantly inhibited the phosphorylation of p27kip1 and the expression of active caspase-3 and cleaved PARP. In addition, flow cytometry analysis also showed that silencing CRM1 by siRNA inhibited TNF-α-induced cellular apoptosis in HT-29 cells.

Conclusions: Up-regulated CRM1 may facilitate IEC apoptosis possibly through p27kip1 in CD, indicating an important role of CRM1 in the pathophysiology of CD.

Introduction

Inflammatory bowel disease (IBD) is a chronic and non-specific inflammatory disease that primarily comprises Crohn's disease (CD) and ulcerative colitis (UC). CD is a chronic relapsing systemic inflammatory disease that may affect any part of the gastrointestinal tract from the mouth to the anus, but especially the distal part of the small intestine (terminal ileitis) and the colon; CD is characterized by bloody diarrhea, abdominal pain, weight loss, and increased risk of gastrointestinal malignancy [1]. Various epidemiological studies have reported that CD is most prevalent in Western industrialized countries [2,3], although the incidence of CD has recently increased in China [4]. Treatment of CD remains a major challenge for clinicians, as no curative therapy currently exists. Thus, marked attention has been paid to the development of new treatments for CD [5]. Although the etiology of CD remains unknown, evidence suggests that genetic, immunological, and environmental factors play roles in the pathogenesis of CD and that intestinal barriers are primary contributing factors [6]. Intestinal epithelial cells (IECs) cover the entire length of the gastrointestinal tract, forming a primary barrier that protects the mucosal surface from harmful molecules and bacteria, and contribute to the regulation of intestinal immune responses [7]. It is known that the balance between epithelial cell apoptosis and proliferation is pivotal for the maintenance of mucosal integrity in the intestine [8]. In CD patients, increased IEC apoptosis has been detected at acute inflammatory sites [9]. However, the mechanisms that contribute to IEC apoptosis in CD remain unknown.

Chromosome region maintenance-1 (CRM1) is the only nuclear exporter protein in the karyopherin-β protein family that contributes to the trafficking of numerous proteins, including tumor suppressor, growth regulator/pro-inflammatory and anti-apoptotic proteins, and RNAs essential for ribosomal biogenesis from the nucleus [10—14]. CRM1 recognizes the leucine-rich nuclear export signal (NES) of cargo proteins that must be shuttled out of the nucleus and then transports them to the cytoplasm [15]. Because nucleocytoplasmic trafficking of proteins/RNAs is essential for normal cellular function, CRM1 has been reported to play crucial roles in the cell cycle, mitosis and replication. Moreover, CRM1 is up-regulated in a variety of solid tumor types, such as gliomas and pancreatic, cervical, and hematological malignancies [16—18]. In fact, abnormally high CRM1 expression is correlated with poor patient prognosis in these malignancies. Therefore, therapeutic targeting CRM1 has emerged as a novel cancer treatment strategy. As reported, CRM1 inhibition can trigger human melanoma cell apoptosis by perturbing multiple cellular pathways [19]. On the other hand, CRM1 has been reported to regulate neuronal apoptosis after traumatic brain injury in adult rats [20]. Therefore, CRM1 is a multifunctional protein, but its expression and potential functions have not been well elucidated in CD.

p27kip1 is a member of the cyclin-dependent kinase (CDK) inhibitor family that acts as a potent tumor suppressor in a variety of human cancers and negatively regulates the transition of cells from the G1 to S phase of the cell cycle, protects against inflammatory injury and promotes epithelial differentiation [21]. Moreover, various studies have shown that p27kip1 is involved in cell differentiation, proliferation, apoptosis, cell-cell adhesion, and growth inhibition [22,23]. Recently, p27kip1 has been shown to be dysregulated in inflammatory bowel disease-associated neoplasia. Loss of p27kip1 protein expression has also been associated with aggressive behavior in IBD-associated neoplasia [21]. As reported, in some cancers, p27kip1 is mislocalized from its cell cycle inhibitory location in the nucleus to the cytoplasm, which changes its role from a tumor suppressor to a tumor promoter [24]. The exact molecular mechanisms responsible for the down-regulation and mislocalization of p27kip1 remain unknown. However, phosphorylation is highly correlated with the mislocalization of p27kip1 to the cytoplasm. Furthermore, CRM1 has been reported to induce the export of p27kip1 from the nucleus, thus promoting DNA damage