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REGULAR ARTICLE

Characterization of endoplasmic reticulum-associated degradation of a protein S mutant identified in a family of quantitative protein S deficiency

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Abstract

Introduction: Misfolded and unassembled glycoproteins are eliminated from the endoplasmic reticulum (ER) lumen by the ER-associated degradation (ERAD).We previously identified a Tyr595Cys (Y595C) mutation of protein S (PS) in a family of a quantitative PS deficiency. The mutation causes intracellular degradation and decreased secretion of the Y595C mutant PS. The aim of the present study was to further characterize the molecular basis of the intracellular degradation of the mutant.
Materials and methods: We stably expressed the mutant in mammalian cells, and analyzed the intracellular localization of the protein. The intracellular degradation pathway was determined by pulse-chase analyses in the presence of various inhibitors of ERAD.

Abbreviations: PS, protein S; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; Man8B, B-isoform of $Man_8GlcNAc_2$; HEK, human embryo kidney; Endo H, Endoglycosidase H; PDI, protein disulfide isomerase; WGA, wheat germ agglutinin; TPPII, tripeptidyl peptidase II; AFF, Ala-Ala-Phe-CH_2Cl.

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Results and conclusions: Endoglycosidase H digestion and immunofluorescence staining revealed the mutant being retained in the ER. Epoxomicin, a potent and specific proteasome inhibitor, and Ala-Ala-Phe-CH_2Cl (AAF), an inhibitor of tripeptidyl peptidase II (TPPII), suppressed the intracellular degradation of the mutant by about 65% and 50%, respectively. When epoxomicin was combined with AAF, the inhibitory effect was substantially enhanced. Although castanospermine, an inhibitor of glucosidases I and II, did not affect the degradation, kifunensine, an inhibitor of ER mannosidase I, suppressed it. Thus, it appears that the Y595C mutant is degraded through more than one pathway of ERAD, including the proteasome-dependent pathway and an alternate proteasome-independent pathway where proteases such as TPPII may be involved. Production of the critical B isoform of $Man_8GlcNAc_2$ targets the mutant for ERAD, however, the interaction with calnexin/calreticulin through monoglucosylated oligosaccharides may not be required for the degradation of the mutant.

Introduction

Protein S (PS) is a vitamin K-dependent plasma glycoprotein that acts as a nonenzymic cofactor to activated protein C in the degradation of factors Va and VIIIa [1]. It consists of 635 amino acids with a molecular mass of ~70 kDa, being composed of multiple domains, including a γ-carboxyglutamic acid domain, a thrombin sensitive region, four epidermal growth factor-like domains and a carboxy-terminal region homologous to sex hormone binding globulin where three N-linked oligosaccharides exist. In human plasma, approximately 60% of the total amount of PS is in an equimolar complex with C4b-binding protein while the remaining free-form PS functions as an activated protein C cofactor. A hereditary deficiency of PS is associated with an increased risk of thromboembolic disease, and various mutations have been identified in the PS gene (PROS1) of deficient patients [2,3]. Studies on recombinant mutant PS expressed in vitro indicate that the difference in phenotype of patients having a quantitative PS deficiency can be ascribed to the severity of the secretion defect of mutant PS [4,5]. However, the precise mechanism of the intracellular degradation of PS mutants has not been identified.

A highly efficient quality control system in the endoplasmic reticulum (ER) operates to eliminate misfolded and incompletely assembled glycoproteins from the ER lumen and destine only properly folded proteins for secretion, for the plasma membrane, and for the secretory and endocytic organelles [6]. Misfolded glycoproteins are retained in the ER by associating with the lectin-like chaperone, calnexin/calreticulin, and selectively retrotranslocated into the cytosol, followed by polyubiquitination and degradation by 26S proteasome, a mechanism known as ER-associated degradation (ERAD) [7,8]. Although the degradation is mainly carried out by the ubiquitin-proteasome system, proteasome-independent proteolysis has been also postulated in the ERAD of several misfolded glycoproteins [9—13].

In addition to promoting protein folding and retaining proteins in the ER, the N-linked oligosaccharide moieties of glycoproteins are considered to be prerequisite to targeting the misfolded proteins for ERAD [14,15]. In particular, reports indicate that formation of the B-isoform of $Man_8GlcNAc_2$ (Man8B) catalyzed by ER mannosidase I is a signal that targets misfolded glycoproteins for ERAD [16—18]. An ER-localized lectin recognizing Man8B glycans, named ER degradation-enhancing α-mannosidase-like protein (EDEM) in mammalian cells [19] and Htm1/Mnl1 in yeast [20,21], have been postulated to promote the release of misfolded glycoproteins from calnexin/calreticulin by functioning as their acceptors [22,23].

By performing a systematic investigation on thrombophilia, we have identified various mutations in the *PROS1* of probands suffering from hereditary PS deficiency [24—28]. A missense mutation, Tyr595Cys (Y595C), has been found in a family of quantitative PS deficiency where the family members heterozygous for the mutation suffered from venous thrombosis with an early age of onset and had a PS deficiency characterized by decreased levels of PS activity, free PS and total PS [28]. Pulse-chase analyses on the Y595C mutant expressed in mammalian cells showed intracellular degradation and decreased secretion of the mutant protein [28]. In this study, we further characterized the molecular basis of the intracellular degradation of the Y595C mutant. The data presented herein showed that the Y595C mutant was retained in the ER of stably transfected mammalian cells and that, although the mutant was mainly degraded by