Molecular Aspects of Medicine 51 (2016) 89–103

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Review

Catabolism of *N*-glycoproteins in mammalian cells: Molecular mechanisms and genetic disorders related to the processes

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ARTICLE INFO

Article history:
Received 18 February 2016
Revised 11 May 2016
Accepted 24 May 2016
Available online 26 May 2016

Keywords:
N-glycan
catabolism
lysosomes
cytosol
genetic disorder
lysosomal storage disease
NGLY1-deficiency

ABSTRACT

N-glycans on glycoproteins serve as one of the most important co- and post-translational modifications of proteins, and it has been well established that they play pivotal roles in controlling the physicochemical and/or physiological properties of the carrier proteins. The biosynthetic/processing pathways for *N*-glycans have been well characterized in mammalian cells. There are, however, issues that remain to be clarified concerning aspects of their degradation. While the molecular mechanism of the lysosomal degradation for *N*-glycoproteins has been well studied in relation to genetic disorders, which are collectively referred to as lysosomal storage disorders, evidence exists to suggest that there are also "non-lysosomal" degradation processes, which are now known to occur widely in eukaryotic cells. In this review, our current knowledge of the lysosomal/non-lysosomal degradation of *N*-glycoproteins in mammalian cells, as well as in human genetic disorders caused by the defects of these processes, is reviewed.

Contents

Abbreviations: AGA, aspartylglucosaminidase; AGU, aspartylglucosaminuria; EBP, elastin-binding protein; ENGase, endo-β-*N*-acetylglucosaminidase; ER, endoplasmic reticulum; ERAD, ER-associated degradation; FNG, free *N*-glycan; KO, knockout; MEF, mouse embryonic fibroblast; PAS, periodic acid-Schiff; PNGase, peptide:*N*-glycanase.

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http://dx.doi.org/10.1016/j.mam.2016.05.004
0098-2997/

1. Introduction

It is widely accepted that lysosomes are the organelle responsible for the catabolism of macromolecules such as proteins, lipids and glycans. Macromolecules that are destined for degradation are delivered to lysosomes either by endocytosis from extracellular spaces or by an autophagic process for intracellular organelles/proteins. Once in the lysosomes, the substrates are degraded by numerous catabolic enzymes. If there is a defect in those enzymes, degradation intermediates can accumulate in lysosomes, resulting in the development of a disorder called a "lysosomal storage disease" (Gieselmann, 1995; Glew et al., 1985; Michalski, 1996; Neufeld, 1991; Winchester, 2005). This fact strongly indicates that our cells do not have the ability to reassemble partially degraded molecules into new functional macromolecules, but degrade them down to monomeric units of sugars and amino acids (or in some cases dipeptides) that are essential for their metabolic re-use process (Aronson and Kuranda, 1989; Winchester, 2005).

In addition to lysosomal degradation, it has been known for some time now that there are cytosolic enzymes (Funakoshi and Suzuki, 2009; Haeuw et al., 1991a) capable of degrading *N*-glycoproteins or their derivatives, free (unconjugated) *N*-glycans (FNGs) (Harada et al., 2015a). While the functional inter-relationships between lysosomal and non-lysosomal degradation are not well understood, it has become clear that both degradation system are critical for normal development in humans.

In this review, I overview the lysosomal/non-lysosomal catabolic processes for *N*-glycoproteins in mammalian cells. A particular focus will be on the enzymes involved in the degradation of the glycan portions of glycoproteins. For more details, readers are directed to the following review articles (lysosomal degradation – Aronson and Kuranda, 1989; Michalski, 1996; Winchester, 2005; non-lysosomal degradation – Harada et al., 2015a; Suzuki, 2007; Suzuki and Harada, 2014).

2. Lysosomal degradation of *N*-glycoproteins in mammalian cells

It has been well documented that the lysosomal catabolism of *N*-glycoproteins occurs in a well-ordered fashion (Aronson and Kuranda, 1989; Michalski, 1996; Winchester, 2005). The pathway has been elucidated by the structural analyses of the storage products in lysosomal storage disease patients, *in vivo* and *in vitro* substrate studies dealing with the substrate specificities of enzymes involved, and the use of the inhibitors to block the action of specific enzyme(s) (Winchester, 2005).

As mentioned above, *N*-glycoproteins destined for degradation in lysosomes are transported to the lysosomes, either from the cell surface or the extracellular space via endocytosis, or alternatively, intracellular delivery involving, for example, the autophagic process (Winchester, 2005). It has been suggested that bulk of proteins, *i.e.* 90% of the long-lived proteins, as well as 80% of the short-lived proteins, undergo lysosomal degradation in the liver in rats (Ahlberg et al., 1985). Once the proteins arrive inside lysosomes, various lysosomal proteases catalyze their degradation. There are a number of different exo- and endoproteases in the lysosomes, and they appear to have overlapping functions (Winchester, 2005). Accordingly, no apparent lysosomal storage disease has been reported due to a lack of a specific lysosomal protease activity (Aronson and Kuranda, 1989; Winchester, 2005).

Proteolysis of the *N*-glycoproteins should occur prior to glycan degradation by various glycosidases, because of the strict substrate specificity of the lysosomal aspartylglucosaminidase; this enzyme requires a free α-amino and an α-carboxyl group on the *N*-glycosylated asparagine as substrates (Kaartinen et al., 1992; Makino et al., 1968; Tanaka et al., 1973; Tarentino and Maley, 1969). The presence of various proteases in lysosomes will therefore ensure that proteolysis is complete and that this facilitates the subsequent glycan catabolism.

3. Enzymes involved in the catabolism of glycan parts of *N*-glycoproteins in the lysosomes

Historically, the lysosomal catabolism of *N*-glycoproteins has been investigated in attempts to clarify the molecular mechanism of the lysosomal storage diseases, collectively called "glycoproteinoses" (Gieselmann, 1995; Michalski, 1996; Neufeld, 1991; Strecker and Montreuil, 1979). In sharp contrast to the case of lysosomal proteolysis, most of the lysosomal degradation of *N*-glycans are catalyzed by a specific enzyme; as a result, a defect in this enzyme can have severe consequences. Glycosidases that are involved in the degradation of *N*-glycans can also be involved in the degradation of other macromolecules; for example, lysosomal sialidase can be involved in the degradation of not only *N*-glycans but also *O*-glycans and glycosphingolipids. In this review, we focus on enzymes that are involved in *N*-glycan catabolism. Figs. 1 and 2 show the proposed degradation pathway for complex-type and high-mannose type glycans, and Table 1 shows genetic disorders that are caused by a defect in enzymes involved in the catabolism of *N*-glycans.

Glycoproteinoses can also be caused by a defect in the mannose-6-phosphate-dependent transport pathway for lysosomal resident proteins (due to the lack of delivery of lysosomal enzymes), but it is beyond the scope of this review