Glycomics & Lipidomics

Extended Abstract

Glycosylated ribosomal protein S3, secreted from various cancer cells is a possible cancer biomarker

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Abstract

Ribosomal protein S3 (rpS3) is a genuine component of the 40S ribosomal small subunit. However, it has been known as a versatile protein with multiple other extra-ribosomal functions in apoptosis, cell cycle control, DNA repair, etc. It has a DNA repair endonuclease activity which is related with various cancers. Recently, we have discovered that this protein forms a dimer and is secreted after N-glycosylation. It is secreted only from various cancer cells but not in normal cells. We also have confirmed that rpS3 is secreted more into media when cancer cells are more invasive. The secretion pathway turned out to be a standard ER-Golgi dependent pathway. We are currently developing various antibodies against rpS3 which could be used as useful reagents for future cancer biomarkers.

Ribosomal protein S3 (rpS3) is a 243 amino acid component of the 40S ribosomal small subunit. It has multiple roles in translation and extra-ribosomal functions like apoptosis and DNA repair. RpS3 is secreted only in cancer cell lines. Presently, mass spectrometry analysis revealed rpS3 to be glycosylated at the Asn165 residue. A point mutation at this residue decreased secretion of rpS3 in cancer cell lines. Secretion was also inhibited by the endoplasmic reticulum (ER)-Golgi transport inhibitor Brefeldin A and by Tunicamycin, an inhibitor of N-linked glycosylation. N-linked glycosylation of rpS3 was confirmed as necessary for rpS3 secretion into culture media via the ER-Golgi dependent pathway. RpS3 bound to Concanavalin A, a carbohydrate binding lectin protein, while treatment with peptide-N-glycosidase F shifted the secreted rpS3 to a lower molecular weight band. In addition, the N165G mutant of rpS3 displayed reduced secretion compared to the wild-type. An *in vitro* binding assay detected rpS3 homodimer formation via the N-terminal region (rpS3:1–85) and a middle region (rpS3:95–158). The results indicate that the Asn 165 residue of rpS3 is a critical site for N-linked glycosylation and passage through the ER-Golgi secretion pathway.

Ribosomal protein S3 (rpS3/*RPS3*/Ribosomal Protein S3) is a constituent of the 40 S ribosomal small subunit, which functions in translation. Extra-ribosomal functions include DNA repair, apoptosis and transcriptional regulation. RpS3 interacts with nm23-H1, which acts as a suppressor of metastasis in certain human tumors and prevents the invasive potential in HT1080 cells. Furthermore, rpS3 is overexpressed in colorectal cancer cells, suggesting that the level of rpS3 may be related to tumorigenesis. A previous study showed that rpS3 was secreted into the extracellular environment in a dimeric form. The level of rpS3 secretion was prominently increased in highly malignant cells when compared to normal parent cells. This suggests that secreted rpS3 may be a putative marker for malignant tumors.

About 10% of all human proteins are secretory proteins. These include cytokines, hormones, digestive enzymes and immunoglobulins. Their various functions include immune defense, intercellular communication, morphogenesis, angiogenesis, apoptosis and cell differentiation. Most of the secretory proteins with amino termini or internal signal sequences are targeted to the cell surface or the extracellular space. The signal sequence is recognized through a signal recognition protein (SRP) and is cleaved once the protein has crossed into the endoplasmic reticulum (ER). The newly synthesized proteins exit the ER and are coated by a cargo-containing coat protein complex II (COPII/SEC23A), targeting them for transport to the Golgi, where they are modified, processed, sorted and dispatched towards their final destination. After passing through the Golgi, secretory proteins are sorted and packaged into post-Golgi transport intermediates, which move to the plasma membrane and fuse with the cell surface.

Post-translational modifications are common in eukaryotic secreted proteins. Protein glycosylation, one of the most abundant post-translational modifications in all organisms, refers to the attachment of saccharide moieties to proteins. Glycosylation participates in protein folding, interaction, stability, mobility, cell adhesion and signal transduction. The glycans of secreted proteins are important for protein secretion, as they influence protein folding, provide ligands for lectin chaperones, contribute to quality control surveillance in the ER and mediate transit and selective protein targeting throughout the secretory pathway. The two major types of glycosylation are N-linked and O-linked glycosylation. Glycans are attached to polypeptide structures through amide linkages to asparagine (Asn) side chains, whereas glycosidic linkages occur with the side chains of serine/threonine (Ser/Thr), hydroxylysine or tyrosine (Tyr), with the latter involving O-

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glycosylation.

Approximately half of all human proteins are glycoproteins, with most containing *N*-glycan structures. *N*-glycans are initially synthesized as lipid-linked oligosaccharide precursors and are transferred from the lipid-linked oligosaccharides to selected Asn residues of the polypeptides that have entered the lumen of the ER. Eukaryotic organisms generally use a multi-subunit oligosaccharyltransferase on the lumenal face of the ER membrane to catalyze glycan transfer to the acceptor peptide sequences, which are comprised of an Asn-X-(Ser/Thr) tripeptide (and less frequently of Asn-X-Cysteine (Cys) and other non-standard sequons), where X can be any amino acid except for proline. Oligosaccharyltransferase facilitates the N-glycosidic linkage between the side chain amide of Asn and the oligosaccharide. Almost all glycans of glycoproteins are subject to trimming and extension as they traverse the Golgi. The present study demonstrates that rpS3 is secreted into the cell culture medium via the ER-Golgi dependent pathway. The secretion, detected using an ELISA assay, can be used as an indicator of cancer cell malignancy *in vitro*. It is also demonstrated that N-linked glycosylation is important for rpS3 secretion and that Asn165 is the site of N-glycosylation, as confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and site directed mutagenesis. Finally, rpS3 forms a homodimer through interactions of the middle and N-terminal regions.

Biography:

Prof. Joon Kim has completed his BS and MS from Seoul National University, PhD in Biochemistry from the University of California at Berkeley and postdoctoral study from Harvard Medical School. He is a Professor in the Division of Life Sciences, and Director of Radiation Safety and Management Center, Korea University, Seoul, Korea. He has published more than 160 papers in reputed journals

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