THE TWO HUMAN ST3GALV ISOFORMS, DERIVED BY ALTERNATIVE TRANSLATION START SITE USAGE, ARE DIFFERENTLY N-GLYCOSYLATED BUT FUNCTIONALLY ACTIVE

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Introduction

hST3GalV (GM3 synthase) is the sialyltransferase that plays a key regulatory role in determining both the cell surface ganglioside profile and various ganglioside-dependent cellular events, including cell proliferation, differentiation, adhesion, apoptosis, oncogenesis. We have reported the identification of an "unusual" hST3GalV mRNA variant, providing the first evidence of the existence of a differentially N-terminal extended isoform of the enzyme and suggesting for it an important role during HL60 cell differentiation (Berselli et al. BBA 2006; 1759, 348-358). The functional relevance of this longer isoform has to be defined yet, and a study to define its sub-cellular localization could provide useful findings to approach this topic.

Methods

To characterize the proteins, c-myc C-terminal tagged hST3GalV isoforms were transiently overexpressed in COS-7 cells and site directed-mutagenesis was used to obtain the selective overexpression of each isoform. The protein N-glycosylation status was verified by PNGase F and endoglycosidase-H treatment, followed by immunoblotting with anti-c-myc-antibodies. The enzyme activity was determined by specific in vitro assays.

Results

In vivo expression of the "unusual" hST3Gal V mRNA variant results in two differentially extended, but both functionally active, hST3Gal V isoforms and mutational analysis demonstrates that these derive from an alternative translation start codon usage. PNGase F and endoglycosidase-H digestions show that, whereas the shorter isoform has both Endo-H sensitive and Endo-H resistant N-glycans, the longer one carries only Endo-H sensitive oligosaccharides.

Conclusions

The different N-glycosylation status does not influence the enzyme activity and provide the first evidence of a different Golgi sub-compartmentalization of hST3GalV isoforms.