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Grant Title: O-GlcNAcylation regulates expression of HAS2 and CD44

ABSTRACT:

The O-GlcNAcylation of the proteins is a dynamic posttranslational modification, which is involved in several biological processes and in human diseases. The O-GlcNAcylation is a reaction able to link N-acetylglucosamine(O-GlcNAc) to the side chain hydroxyl group of a serine or threonine residue. In recent years, in several human malignant tumors the metastatic potential was linked to O-GlcNAcylation. Hyaluronan (HA) is an unbranched and unsulphated glycosaminoglycan synthesized in the cells by specific membrane synthases (HAS 1, 2 and 3). HA synthesis is often related to cancer progression and metastatic potential. We found that HAS2 could be O-GlcNAcylated at serine 221 increasing its stability in the cell membrane and its capacity to produce HA (1). CD44 is a HA receptor and represents a signaling platform that integrates cellular microenvironmental cues with growth factor and cytokine action transducing signals. Accumulating evidence indicates that CD44, especially CD44v isoforms, are cancer stem cell (CSC) markers and critical players in regulating the properties of CSCs.

Objectives:

In the study, we would like to demonstrate that O-GlcNAcylation triggers the HAS2 expression at epigenetic level (2) and increases the expression of its antisense HAS2-AS1 and this can be coupled with CD44 expression.

Methods:

For the study, we used human smooth muscle cells and breast cancer MCF7 (low malignant) and MDA-MB-231 (triple negative highly malignant) in vitro. The cell have been transfected by using si RNAs coding for different genes and lncRNA HAS2-AS1, a long non coding RNA that we found involved in our model. Cell viability was assessed by MTT and the cell migration with scratch test and matrigel has been used for cell invasion test. Gene expression was measured by quantitative RT-PCR with specific TaqMan probes.

Results:

After the O-GlcNAcylation induction by glucosamine incubation, we previously found that HAS2 antisense (HAS2-AS1) expression was highly increased due to the p65 O-GlcNAcylation, indicating that the antisense was induced by NFkB pathway (3). In breast cancer cells HAS2-AS1 is expressed as long and short forms, both highly expressed in tumours correlating with cell aggressiveness. The modulation of HAS2-AS1 affects the cancer cell behaviour and its knock down increased the cancer cell invasion and viability. On the other hand, the overexpression of the two isoforms of HAS2-AS1 (HAS2-AS1 long being the more effective) decreased cell viability and their ability to penetrate Matrigel. The abrogation of HAS2-AS1 induced a higher expression of HAS2 and HAS3 mRNA, as well as increased the hyaluronidase 2 transcript levels. The treatment brought to an increase of CD44 mRNA levels. Although we observed an increase of CD44 transcript, there was not an activation of the p44/42 pathway (ERK1/2 pathway). These data indicate that HAS2-AS1 could globally control HA metabolism, not only through the expression of its synthases but also through its receptor and degrading enzymes.

1: Vigetti D et al. Role of UDP-N-acetylglucosamine (GlcNAc) and O-GlcNAcylation of hyaluronan synthase 2 in the control of chondroitin sulfate and hyaluronan synthesis. J Biol Chem. 2012 Oct 12; 287(42):35544-55.

2: Vigetti D et al. Epigenetics in extracellular matrix remodeling and hyaluronan metabolism. FEBS J. 2014 Nov; 281(22):4980-92

3: Vigetti et al. Natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) induces transcription of HAS2 via protein O-GlcNAcylation. JBC 289, 28816-28826