Production of the extracellular domain of Siglec-9 using an E.coli expression system and generation of anti-Siglec-9 antibodies

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INTRODUCTION:

• Sialic acid-binding immunoglobulin-like lectins (Siglection) are a subset of the immunoglobulin superfamily of cell surface receptors.

• Siglection mediates protein–carbohydrate interactions through their ability to bind sialic acid moieties found on glycolipids and glycoproteins.

• Siglections are mainly expressed on white blood cells and play a critical role in cell-cell interactions and signaling functions in the hematopoietic, immune, and nervous systems.

• The human Siglection family is composed of 11 genes. In addition to well-characterized Siglections such as the myeloid receptor (Siglection-3CD33), seven additional Siglections (Siglection-5-11) have been identified in recent years.

• All newly identified Siglections have a high degree of sequence similarity to Siglection-3 and are collectively referred to as Siglection-3-related genes.

• Anti-CD33/Siglection-3 antibodies have recently been used for diagnosis of acute myeloid leukemia (AML).

• The development of Siglection-3 antibody-targeted chemotherapy, as well as emergence of anti-Siglection-5 antibodies as a potential marker and therapeutic reagent for AML, has prompted the characterization of other newly identified Siglections.

• Siglection-9 is one of the newly identified members of the Siglection gene family.

• Similar to other Siglection-3-related genes, Siglection-9 is located on chromosome 19q 13.3-4 in close proximity to the kalirin gene locus.

• Siglection-9 is comprised of N-terminal V-set Ig domains that mediates sialic acid binding, two C2-set Ig domains, a transmembrane region and a cytoplasmic tail that harbors two immune receptor tyrosine-based inhibition motifs (ITIMs).

• The Siglection-9 protein is expressed in bone marrow, spleen, placenta, and fetal liver. Its expression in tissues involved in stem-cell differentiation indicates that Siglection-9 may play a role in the activation of several cell types and hence the regulation of tumor growth.

• Siglection-9 protein is composed of 463 amino acids harboring an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

• The extracellular domain of Siglection-9 is composed of 321 amino acids with a molecular weight of 35 kD.

• Here we report the production of the extracellular domain of Siglection-9 using an E.coli protein expression system.

• This domain was expressed as a fusion protein containing an N-terminal polyhistidine (6xHis) tag.

• Recombinant Siglection-9 was purified to homogeneity using metal affinity chromatography and was used as immunogen for antibody production in rabbit and mice.

METHODOLOGY:

• Commercially available total bone marrow tissue mRNA was reverse-transcribed to cDNA.

• Polymerase chain reaction was conducted using the proof reading enzyme Pfu and oligonucleotide primers specific to the extracellular domain of Siglection-9.

• The amplified cDNA was purified and cloned into pET200 TOPO plasmid vector containing an N-terminal polyhistidine (6xHis) tag.

• Plasmid DNA containing the pET-Siglection construct was isolated and sequenced to confirm its identity and then was used to transform the E.coli strain BL21(DE3) for protein production.

• Protein expression was induced with isopropyl thio galactoside (IPTG), and E.coli culture was harvested 3 hours post IPTG induction.

• Whole cell extracts of cultured BL21 cells were subjected to SDS-PAGE and Western blotting.

• The most prominent band observed in gels stained with Coomassie blue corresponded to the expected molecular weight for Siglection-9.

• Western blots using anti-histidine antibody resulted in detection of a single band.

• The identity of the Siglection-9 fusion protein was confirmed by mass spectrometry.

• Siglection-9 was mainly produced in "inclusion bodies". The inclusion bodies were isolated, washed with 2 molar urea, and then dissolved with guanidine hydrochloride.

• The recombinant protein was purified to homogeneity using nickel-nitrilotriacetic (Ni-NTA) metal affinity chromatography.

• Siglection-9 was used as immunogen for production of antibodies in New Zealand White rabbits and female BALB/c mice.

• Specific affinity of anti-Siglection-9 antibodies was tested using antibody capture assays and Western blotting.

RESULTS:

Fig. 2 Detection of recombinant protein expression as detected by Western blotting using Anti-his tag (HisG-AP) antibody

Lane 1. See Blue Marker
Lane 2. BL21 E. coli cell lysate (vector only)
Lane 3. BL21 E. coli cell lysate (Siglection insert + IPTG)
Lane 4. Blank
Lane 5. Purified Siglection-9

DISCUSSION:

• In this study the extracellular domain of Siglection-9 was expressed as a fusion protein using an E.coli protein expression system.

• The expected molecular weight for the extracellular domain of Siglection-9 is 35 kD. In our experiments the apparent molecular weight as observed in figure 2 is slightly higher, this is due to the presence of the 3 kD N-terminal tag.

• Purified Siglection-9 was used as an immunogen and polyclonal and monoclonal antibodies were generated.

• Polyclonal and monoclonal antibodies generated were highly specific to Siglection-9 as assessed by Western blot analysis (Figure 3) and antibody capture assays (data not shown).

• Polyclonal anti-Siglection-9 antibody was used in flow-cytometric analysis of lymph nodes obtained from patients with hematological malignancies. 7 out of 8 patients with B-cell lymphoma were positive.

CONCLUSION:

• Our results indicate that that Siglection-9 may be a new surface marker of patients with B-cell lymphoma.

• We are currently using the Siglection-9 specific antibodies to develop a sandwich ELISA capable of measuring the levels of this Siglection in various biological fluids.

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