**Principal Investigator: Hiroto Katoh**

**Grant Title: Elucidation of the biology of sulfated-GAG using human antibody library.**

## (a) Abstract

### 1. Objective

In my previous research, it had been elucidated, rather unexpectedly, through next-generation sequencing analysis of immunoglobulin genes of tumor-infiltrating B cells, that "anti-sulfated glycosaminoglycan antibodies" exist abundantly in human cancer tissues (Cell Reports 2017, Communications Biology 2020). Sulfated glycosaminoglycans, as proteoglycans, interact with growth factors, cytokines, their receptors, and extracellular matrices, and are known to control cell growth, adhesion, and migration. It is thought that sulfated glycosaminoglycans are essential for life, as knockout mice of genes involved in their biosynthesis show fetal lethality. However, the detailed biological significance of sulfated glycosaminoglycans *in vivo* is unknown. It is known that there is some variation in the degree of sulfation in each sulfated saccharide, and it is thought that the substances collectively referred to as "sulfated glycosaminoglycans" contain a vast repertoire of functional diversity. To date, little is known about the distribution of diverse sulfated glycosaminoglycans *in vivo*, their fine localization in each cell type, their relationship with the stromal composition, and their biological significance.

### 2. Methods

In this study, the first objective was to detail the antigen epitope structures of the "anti-sulfated glycosaminoglycan human antibodies" isolated from next-generation sequencing analysis of immunoglobulin genes (antibody genes) of human cancer tissues, by establishing a unique sulfated saccharide library, and to identify saccharide structures that serve as functional cancer antigens among structures of diverse sulfated saccharides. Furthermore, by performing immunostaining and cell staining of human cancer tissues using these human antibodies, we aimed to elucidate the distribution of specific sulfated saccharide structures *in vivo*, their fine localization in cells, and the biological significance of sulfated saccharides in cancer biology.

### 3. Results

The results of this study revealed that the antigens recognized by the anti-sulfated glycosaminoglycan human antibodies, which were discovered in human cancer tissues, are “densely-sulfated glycosaminoglycans”. Interestingly, it was found that these anti-densely-sulfated glycosaminoglycan human antibodies show a significantly enhanced antigen reactivity specifically in lower pH environments that mimic cancer environments. Furthermore, when immunostaining of human cancer tissues was performed, it was revealed that multiple anti-densely-sulfated glycosaminoglycan human antibodies isolated from the human cancer environment show specific and broad reactivity to human cancer cells in various tissues. These findings suggest the potential application of the anti-densely-sulfated glycosaminoglycans human antibodies identified in human cancer environments as therapeutic antibodies for cancer treatment, which is considered to be a very interesting outcome of this research.