Structure, diversity, and function of the membrane-bound complement regulatory protein in ginbuna crucian carp Carassius auratus langsdorfii

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（ギンブナ膜結合型補体制御タンパク質の構造、多様性および機能に関する研究）

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論　文　内　容　の　要　旨

Regulators of complement activation (RCA) are classified into the soluble and membrane-bound proteins, and regarded as to play a key role in protecting host cells from excessive complement activation in mammals. In bony fish, those two classes of RCA have also been found. However, functions of membrane-bound RCA protein remain to be characterized. Recently, complement regulatory membrane proteins (Tecrem) has been found in carp and zebrafish. The present study aimed at identifying Tecrem orthologues in ginbuna crucian carp, and analyzed their diversity, expression, and function at mRNA and protein levels.

For identification of Tecrem cDNA from ginbuna crucian carp, a primer, corresponding to a sequence containing the predicted 5'-untranslated region, was designed on the basis of the Tecrem sequence from common carp. 3'-RACE PCR was performed and the amplified cDNA was sequenced. As a result, three isoforms of membrane-bound RCA have been identified in ginbuna crucian carp (gTecrem-1, gTecrem-2, and g-Tecrem3).

Expression analysis at mRNA level was performed using total RNA extracted from eleven tissues and blood cells (erythrocytes, total leucocytes, $CD4^+$ T cells $CD8^+$ T cells and IgM^+ B cells), which were subjected to RT-PCR. The gTecrem isoforms showed different mRNA expression patterns; Only gTecrem-1 mRNA was expressed in both peripheral blood leukocytes (PBLs) and erythrocytes and was also expressed in T cell subsets. Moreover, gTecrem-1 has a tyrosine phosphorylation site in its cytoplasmic tail, while other isoforms lack its site.

To perform functional analysis at the protein level, monoclonal antibody specific for carp Tecrem (anti-Tecrem mAb) was established and shown to cross-react Tecrem of ginbuna crucian carp (gTecrem). Expression of gTecrem on leucocyte and erythrocyte was determined by FCM analysis using anti-Tecrem mAb. To understand whether gTecrem is involved in T cell immunity, we investigated regulation of gTecrem on PBL after stimulation with PHA, a known T cell mitogen. Furthermore, effect if anti-Tecrem mAb on proliferation of PBL triggered by the PHA-stimulation was examined by MTT assay.

gTecrem protein was detected on both erythrocyte and leucocyte. Expression of gTecrem on PBL was upregulated following stimulation with PHA. The proliferative response was inhibited by addition of anti-Tecrem mAb, suggesting that gTecrem are involved in T cell activation.

In conclusion, three diverged isoforms of membrane-bound RCA have been identified in ginbuna crucian carp. gTecrem-1 was suggested to be functionally equivalent to mammalian CD46, in that CD46 is expressed on T cells and promotes T cell activation.