Development and characterization of an anti-diacylglycerol kinase zeta monoclonal antibody for immunocytochemistry

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Purpose: Diacylglycerol kinase (DGK) is an enzyme that converts diacylglycerol (DG) to phosphatidic acid (PA). As both DG and PA serve as lipidic second messengers, DGK plays a pivotal role in controlling the balance of two signaling pathways mediated by DG and PA in cellular functions. DGKζ, one member of the mammalian DGK family, is reported to contain a nuclear localization signal, which suggests its functional role in the nucleus. In this study, we aimed to develop anti-human DGKζ monoclonal antibodies (mAbs), which are useful for immunocytochemistry (ICC) of human cultured cells.

Methods: Rats were immunized by injecting mouse DGKζ recombinant protein, and hybridoma cells were produced by the fusion between myeloma cells and lymphocytes from medial iliac lymph node. Anti-DGKζ mAbs were screened by using enzyme-linked immunosorbent assay, western blot (WB), and ICC analyses. The binding epitope of anti-DGKζ antibodies were determined using WB.

Results: One of anti-DGKζ mAbs, DzMab-1 specifically detected DGKζ, and did not recognize DGKα and DGKγ in WB. DzMab-1 is also useful in ICC analysis of HeLa cells. Epitope mapping demonstrated that Met1 and Pro3 residues of human DGKζ is important for the binding of DzMab-1 to DGKζ.

Conclusion: DzMab-1 could be advantageous for ICC analyses for human DGKζ of human cell lines.