

Creation of valuable antibodies by an *in vitro* antibody generation system using a hypermutating B cell line

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Monoclonal antibodies (mAb) have recently proven to be excellent biopharmaceutical agents. The generation of hybridomas from antigen-stimulated B cells has been a key technology for obtaining mAbs; however, it is a laborious and time-consuming process, and sometimes mAbs for molecules conserved between species are difficult to be obtained because of immunological tolerance. Thus, it is of great importance to develop *in vitro* technologies for generating useful Abs as drug candidates. We have been challenging to develop a novel *in vitro* antibody generation system using a chicken B cell line DT40, which displays Abs and mutates Ig genes by point mutation and gene conversion during culture, thereby generating a useful Ab library for screening mAbs. First, we generated an engineered cell line DT40-SW whose mutation machinery can be reversibly switched on and off. The Ab generation system using DT40-SW was found to be useful as follows: (1) mAbs for various model antigens including autoantigens can be obtained from DT40-SW Ab library that is free from immunological tolerance; (2) the switching device of the mutation machinery enables to fix desirable Ig mutants by stopping mutation or to resume mutation for further improvement of Ab affinity; (3) by repeated culture and sorting of clones bearing higher affinity for target antigens, affinity maturation can be mimicked *in vitro*. For practical application in obtaining valuable Abs such as antitumor Abs, the Ab generation system is undergoing the improvement of mutation efficiency, selection method and Ab production, and the development of a platform for affinity maturation of given Abs in DT40.