Generation of Anti-Steroid Antibody with High Affinity Based on Antibody Engineering

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Immunoassays have been an essential tool for trace characterization of endogenous steroids. To improve the sensitivity of the steroid immunoassays, anti-steroid antibodies showing much higher affinity than that of conventional antibodies are required. Recent progress of antibody engineering offers promising strategies for generating artificial antibody fragments (*e.g.*, single-chain Fv fragments (scFvs) that are composed of V_H and V_L domains combined via a flexible linker peptide) that possess higher affinity and/or specificity. In a typical protocol, highly diversified mutated *scFv* genes are expressed on the filamentous phage particles, from which the "phage-scFvs" with improved binding characteristics are isolated by a procedure called biopanning. The selected scFvs on the phage can be prepared in a soluble form, being detached from the phage particles, which is suitable for various immunochemical applications. Such a mutated gene library can be constructed by introducing random mutations into V_H and/or V_L gene(s) in an arbitrarily selected *scFv* gene. Complementarity-determining regions (CDRs) have been considered to be the target sites for such mutagenesis that alters the binding property of the original antibody in a high probability. We have constructed a mutated phage-scFv library based on the "CDR-shuffling" technique, in which 4 kinds of mutated *CDR*-gene fragments, the original sequence of which encodes V_H-CDR2, V_H-CDR3, V_L-CDR1 or V_L-CDR3 of a mouse anti-estradiol-17β (E_2) antibody, had been incorporated in random combinations to make a large diversity. From this library, a phage-scFv clone, the soluble scFv from which shows *ca.* 10-fold higher affinity to E_2 than that of the mouse antibody, has been isolated.