

Development of monoclonal antibodies able to distinguish between wild-type and mutant FANCC proteins by a systematic epitope scanning approach

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FANCC is a 558 amino acid protein of unknown function. Inactivating mutations have been described in *FANCC*, including a transition resulting in a proline to leucine substitution at residue 554 (L554P). Based on a systematic analysis of epitopes recognized by FANCC-specific polyclonal antibodies, we developed a panel of monoclonal antibodies that recognize wild-type FANCC in immunofluorescent, immunoblot and flow cytometric analyses, but that do not recognize the L554P mutant protein. Epitope mapping showed that these monoclonal antibodies recognize a linear epitope at the carboxyl terminus of the FANCC protein. Monoclonal antibodies were used in IP-western blot experiments to screen cell lines containing *FANCC* mutations, including EUFA450 (heterozygous for 322delG and L554P). This cell line is MMC hypersensitive, but can convert to MMC resistance by reversion. The monoclonal antibody 8F3 was able to clearly distinguish cell lines containing wild type FANCC, including the appearance of a new band corresponding to wild-type FANCC in the reverted cell line. These results suggest that the epitope selection method is a successful approach for producing a useful monoclonal antibody from a poorly antigenic protein.