Chapter 6

Cloning and molecular characterization of monoclonal antibody-defined ovarian tumour antigens

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6.1 INTRODUCTION

In an effort to develop additional treatments and improved methods for early detection of ovarian cancers a number of monoclonal antibodies (MAbs) have been generated against cell surface antigens of epithelial ovarian cancer (EOC). Therapies using these reagents to target ovarian cancers have so far been disappointing. Identifying the antigens recognized by the antibody may be important for improving the targeting of antibody based therapy. For example, detailed molecular analysis of the polymorphic epithelial mucin gene, MUC1, has shown that differential glycosylation of a tandem repeat unit is responsible for the appearance of novel epitopes in breast, ovarian and other adenocarcinomas [1]. This information has opened up new possibilities for immunotherapy with mucins since these structures are highly immunogenic and are relatively tumour specific. The encouraging results of such studies prompted us to undertake the cloning of the genes for other MAb defined tumour antigens based on an expression cloning technique [2]. In this chapter we briefly summarize the important characteristics of the genes cloned so far and discuss their possible function in ovarian cancer.

6.2 CLONING STRATEGY AND RESULTS

All the cDNAs were cloned using an expression cloning system described in detail previously [3,4] and the overall strategy is outlined in Figure 6.1. After each MAb specific clone was isolated, its identity as the in vivo target was confirmed either by comparison of the size of products produced by transfectants with that derived from cell lines and/or by correlation of cell surface reactivity with the presence of corresponding levels of mRNA. To ascertain if mutation was the basis for tumour specific reactivity of the MAb the sequences of cDNAs from carcinoma cell lines were then compared with those derived from normal tissues. The deduced amino acid sequence was used to search for homologous proteins in EMBL and Swiss protein databases and to analyse the protein for any distinctive structural motifs. Finally, the chromosomal localization of the gene was determined using fluorescence in situ hybridization and by molecular techniques. The involvement of the region in chromosomal aberrations such as allele loss, amplification or rearrangement was investigated in tumour and normal DNA from a collection of 30 ovarian tumours. By combining these techniques we hoped to be
Figure 6.1 Project strategy. Abbreviations: YAC, yeast artificial chromosome; PFG, pulse field gel; RFLP, restriction fragment length polymorphism; VNTR, variable number tandem repeat.