



Tissue microarrays: Applications in study of p16 and p53 alterations in Ewing's cell lines

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Abstract

Background: Tissue microarrays (TMAs) are used to study genomics and proteomics in several tumour tissue samples. Cell lines (CC) are of great importance in the study of the genetic changes in tumours, and some reveal several aspects of tumour oncogenesis. There are few published reports on Ewing's tumours with TMAs including original tumours (OT) and corresponding CC.

Methods: We have performed four TMAs, from 3 OT and the corresponding CC of successive *in vivo* and *in vitro* tumour passages. Xenotransplant CC in nude mice from OT (XT/OT) was made. Subsequently multiple XT were performed and *in vitro* XT cell line (CC/XT) was obtained. *In vivo* re-inoculation of CC/XT (XT/CC) was planned. TMAs with the successive tumour passages that grew in nude mice (XT/OT and XT/CC) were analyzed by morphologic pattern (Hematoxylin/eosin), immunohistochemical staining (CD99, FLII, p16, p53, ki-67), fluorescent *in situ* hybridization- FISH- (EWSR1 break apart, p16 and p53 status) and gene fusion types.

Results: Heterogeneous results of the p16, p53 and ki67 in OT, XT/OT, CC/XT and XT/CC were observed. The three cell lines revealed EWS/FLII rearrangements. p16 gene was deleted only in one case. The deletion was detected by FISH and confirmed by PCR assays. A p53 alteration was found in the second case with monosomy and subsequently polysomic status of chromosome 17 during the evolution of CC. The PCR study revealed p53 mutation. The third case showed hypermethylation in the promoter of p16. The growth of the tumour in nude mice was more accelerated when the inoculation was performed from the CC/XT, increasing progressively over the passages. The third case did not reveal tumour growth in nude mice after the re-inoculation of CC/XT.

Conclusions: The study of several cores from original tumours and successive tumour passages in TMAs facilitated the analysis of the genetic alteration and protein expression in Ewing's tumours.

Keywords: TMAs, Ewing/PNET cell lines, xenotransplant tumors, p16-p53 alterations