

Supplementary Materials and Methods

Cell cultures, DNA isolation, and xenografts

Except KYAE-1, all cell lines were cultured under standard conditions in RPMI-1640, supplemented with 10% Fetal Calf Serum. Cell line KYAE-1 was cultured in Ham's F12/RPMI-1640 1:1 with the addition of 10% fetal calf serum. To verify the histomorphological characteristics of each cell line, 5×10^6 tumor cells were injected subcutaneously in two female NMRI nude mice (Taconic-M&B, Ry, Denmark) or two NOD-SCID mice (in-house breeding). Xenograft tumors were removed after reaching a visible size of 0.5–1 cm in diameter and were routinely processed for histological and immunohistochemical examination (10). The mouse experiments were licensed and done in accordance with approved protocols by the Erasmus MC, University Medical Center Rotterdam, the Netherlands. DNA was isolated from cultured cells, original tissues, and xenograft tumors using the PureGene Genomic DNA isolation Kit (Qiagen, Venlo, The Netherlands). DNA was diluted to a concentration of 100 ng/ μ L to perform polymerase chain reaction (PCR).

Short Tandem Repeat (STR) profiling

All cell lines were genotyped by STR profiling by using the Powerplex 16 System (Promega Madison WI). The Powerplex 16 system comprises fifteen STR loci, including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317, D5S818, and the sex chromosome marker Amelogenin. Amplification was performed using 1 ng of template DNA applying the

Powerplex 16 system following the manufacturer's recommendation. Multiplex PCRs were carried out with fluorescent dye-linked primers. Labelled products were detected by electrophoretic size fractionation on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). The data were analyzed by using Genemarker software (SoftGenetics LLC, State College, PA) to categorize peaks according to their size in relation to an internal standard. This analysis enabled every peak to be allocated a size corresponding to the number of repeat units present.

Inability to amplify the larger STR fragments of DNA isolated from paraffin embedded archival tissues (due to degraded DNA) forced us to use PCR primers closer to the repeat structures in order to perform single STR profiling. Using this method, eight single STR loci were investigated in the original tissue and in the corresponding cell line. The size of DNA fragments found in the cell lines could be linked to the number of repeats known from the Powerplex assay. This enabled us to correlate the length of the DNA fragments found in the original tissues to the number of repeats.

Mutation analyses

Because TP53 is frequently mutated in EAC, all exons and intron-exon boundaries of the *TP53* gene were commercially sequenced in all the EAC cell lines (Asper Biotech Ltd, Tartu). The detected *TP53* mutations were investigated in the primary tissues from the cell lines were derived, by in-house sequencing at the Department of Pathology, Erasmus MC, University Medical Center Rotterdam.