Appendix S1

SUPPLEMENTARY MATERIAL AND METHODS

Sample preparation and DNA extraction

An apocrine carcinoma, basal cell carcinoma, and sebaceous naevus were pathologically identified and dissected from formalin-fixed paraffin-embedded tissue sections macroscopically or microscopically by laser microdissection using a Zeiss PALM MicroBeam IV Laser-Captured Microdissection system (Carl Zeiss Microscopy GmbH, Göttingen, Germany). DNA was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). Extracted DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA).

Sanger sequencing

DNA (10 ng) was amplified and purified from agarose gel, followed by direct sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For these procedures, the following primers were used: *HRAS* exon 2 forward primer: GGA GAC GTG CCT GTT GGA and *HRAS* exon 2 reverse primer: GGT GGA TGT CCT CAA AAG AC.

Immunostaining

FFPE sections (4-μm thick) from representative blocks were deparaffinized and rehydrated in graded alcohols and distilled water. Immunostaining for p63 was performed on a fully automated slide preparation system (Benchmark XT System, Ventana Medical Systems, Tucson, AZ, USA) with anti-p63 monoclonal antibody (Epitomics, Burlingame, CA, USA) at a concentration of 1:200 dilution overnight at 4°C. Prior to staining, antigen retrieval was performed using a microwave for 25 min. For Ras G13R staining, a peroxidase block (DAKO, Glostrup, Denmark) was carried out for 5 min. For antigen retrieval, heating was performed in 10 mmol/L sodium citrate, pH 6.0. The sections were incubated overnight at 4°C with anti-Ras G13R monoclonal antibody (NewEast Biosciences, Malvern, PA, USA), which was followed by incubation with a secondary antibody (EnVision Detection System, DAKO). The staining was developed with 3-amino-9-ethylcarbazole substrate (DAKO) and counterstained with haematoxylin.