**Supplementary table 1.** Top tipifarnib modulated genes used for ENRICHR GSEA.

**Supplemental Materials and Methods**

**Cell lines and tissue culture**

Human head and neck cancer cell lines CAL27, Detroit (*HRAS* wild type), HN31 and UMSCC17B (*HRAS* mutant) were collected as part of the NIDCR Oral and Pharyngeal Cancer Branch cell collection and have been described previously [1] [2]. The novel cell lines ORL48 (*HRAS* wild type) and ORL214 (*HRAS* mutant) were generously provided by Dr. Sok Ching Cheong [3]. To ensure consistency in cell identity, all the cell lines underwent DNA authentication by multiplex STR profiling (Genetica DNA Laboratories, Inc. Burlington, NC, USA) prior to experiments. No mycoplasma was detected through Mycoplasma Detection Kit-QuickTest from Biomake (Houston, TX, USA). All the cell lines were cultured using Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and antibiotic solution at 37ºC in the presence of 5% CO2.

**Tipifarnib**

Tipifarnib (provided by Kura Oncology) was synthesized as previously described [4] and stored as a powder at room temperature or as 10mM DMSO stock at -80 oC. For *in vivo* use, the drug was formulated at 8mg/ml in 20% w/v hydroxypropyl-β-cyclodextrin, pH 2.5. Formulated drug was prepared once weekly and stored at 4 oC and protected from light.

**RNA interference treatment**

In order to achieve siRNA-induced knockdown of HRAS, siRNA SMART-pool targeting HRAS (Dharmacon- L-004142-00-0005) and Non-targeting siRNA (Dharmacon D-001206-13-05) were transfected using RNAimax (Invitrogen) as described in the manufacturer's instructions.

**Cell growth assays**

Alamar Blue Cell Viability Reagent was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cells were cultured in 96-well plates and treated with tipifarnib or DMSO as a control for 48h. The manufacturer's instructions were followed to complete the assay.

**3D spheroids assay**

Cells were cultured in 96-well-plates in 1% agar and treated with tipifarnib or DMSO as a control for three weeks. Tipifarnib was replenished every week. For proliferation analysis, Alamar Blue Cell Viability Reagent was purchased from Thermo Fisher Scientific (Waltham, MA, USA) and the manufacturer's instructions were followed to complete the assay. The number of spheroids was quantified by Imaging (Image J software)[5].

**Immunoblot analysis**

Cells were lysed in RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA, USA), and Western blot assays were performed as described [2]. Antibodies used were from Cell Signaling Technology (Danvers, MA, USA), pERK (catalog number 4370), ERK (catalog number 9106), pMEK (catalog number 9122 CST), MEK (catalog number 9154 CST), GAPDH (catalog number 2118), HRAS (Abcam catalog number ab97488) and GFP (Covance, catalog number MMS-118R).

***In vivo* mouse experiments and analysis**

Studies on cell line-derived HNSCC xenografts were performed at the University of California, San Diego under protocol ASP # S15195, approved by the Institutional Animal Care and Use Committee (IACUC). Female athymic nu/nu and NOD SCID mice (4–6 weeks of age) were obtained from Charles River Laboratories (Worcester, MA, USA). HNSCC cells (2 million/tumor) were transplanted into both flanks. When tumor volume reached approximately 150-200 mm3, mice were randomized into groups and treated with vehicle diluent or tipifarnib (60mg/kg BID) for approximately 20 days. Tumor size was measured by caliper twice weekly in two dimensions. The tumor volume was expressed in mm3 using the formula: TV = 0.5 a × b2 where a and b are the long and short diameters of the tumor, respectively. Body weight was measured twice per week. The mice were euthanized at the end of experiments, and tumors were isolated for histologic and immune-histochemical evaluation.PDX studies were carried out at CrownBio. The protocol and any amendment(s) or procedures involving the care and use of animals were approved by the Institutional Animal IACUC of CrownBio prior to conduct. During the study, the care and use of animals was conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The HNSCC PDX models used were HN1420, HN2579, HN2581, HN3504 (*HRAS*-mutant) and HN0626, HN2609, HN3411, HN5111, HN5115 and HN5123 (*HRAS* wild-type). HN0626, HN1420, HN3411 and HN3504 are of Asian origin, the remainder are of Caucasian origin. BALB/c nu/nu mice were inoculated subcutaneously with 2-3 mm tumors fragments, the PDX were allowed to establish to 250-350 mm3, the animals were randomized into groups of three and treated orally BID with vehicle or tipifarnib as described above.

**Immunohistochemistry and immunofluorescence of paraffin embedded tissues:**

Immunohistochemical analysis of pERK, Cleaved Caspase-3 were performed following our previously reported procedures [6]. The following antibodies were used: pERK (catalog number 4370), Cleaved Caspase-3 (catalog number 9661). All tissue samples were processed and stained as previously described [7]. All tumors were harvested and processed. Paraffin embedded slides were stained using anti CD31 (BD Pharmingen 550274), anti-farnesyl antibody (Millipore, AB4073), anti KI67 antibody (Abcam, 156956) and KRT4 (Abcam 51599) Samples were mounted in prolong gold anti-fade mounting medium (Invitrogen) and scanned with Axioscan Z1 (Zeiss) and analyzed using QuPath [8] software or visualized inverted Zeiss LSM 780 confocal microscope coupled to Zen software (Carl Zeiss).

**HRAS plasma membrane translocation assays:**

For HRAS-GFP transfection, cells were grown on μslide glass bottom (Ibidi). Cells were transfected with HRAS-GFP and the next day were treated with tipifarnib for 48h and placed in the microscope for the image acquisition. The imaging analysis and quantification were performed by means of QuPath software [8].

**Microfluidic vasculogenesis assay**:

GFP HUVECs passage5 from Lonza 4M/ml were seeded through microfluidic channels in a 3D environment as previously described [9] and treated with tipifarnib 200 nM for 48h. Samples were fixed in PFA 4% and imaged through 780 confocal microscopy and the number of branches were quantified in at least three ROIs for each condition, as previously reported [10]. at least 3microfluidic device for each condition have been cultured. The experiment has been repeated 3 times (n=3)

**Mouse Choroidal Explant Assay**

Male C57BL/6J mice (age P20) were euthanized and eyes were immediately enucleated for dissection. After removing the cornea and lens, the peripheral choroid-scleral complex was separated from the retina and cut into approximately 1mm x 1mm fragments, essentially as previously described [31]. After adding 60 µL of growth factor-reduced Matrigel (Corning, #354230) to each well in 48-well plates, peripheral choroid-scleral fragments were placed in the center of each well and covered with a top layer of 60 µL of Matrigel. Plates were incubated at 37C for 30 minutes to allow the Matrigel to solidify. Five hundred µL of growth medium (Endothelial Cell Growth Basal Medium (EBM-2, Lonza, #CC3156) supplemented with 2% FBS and Pen-Strep) were then added to each well along with 300 nM Tipifarnib or DMSO control. Tissues were placed in an incubator at 37 °C and media were changed every 48 hours. Phase-contrast Z-stack images of each explant were taken on day 4, 5 and 6 using a Keyence microscope. Vessel sprouting areas were quantified using ImageJ. Data were obtained analyzing 6 replicates per each condition from three independent experiments [11].

**RNA sequencing and bioinformatic analysis of PDX response to tipifarnib**

HN2579 and HN3504 HNSCC PDX tumors were implanted in groups of three animals as described above and allowed to grow to 350-450mm3, treated for four days with vehicle or tipifarnib (80mg/kg BID), excised and snap-frozen. Three fragments were microdissected from different regions of the tumors into to ensure unbiased sampling of the tumor masses. RNA was extracted from cells using the QIAGEN RNeasy mini kit. RNA library preparations and sequencing reactions were conducted at GENEWIZ, Inc (South Plainfield, NJ). The NEBNext Ultra RNA library Prep kit (New England BioLabs) was used for RNA library preparation. In brief, poly(A) mRNA was enriched with oligo d(T) beads and fragmented (15 min at 94oC). cDNA fragments were subsequently synthesized, end repaired and adenylated at 3’ends, followed by universal adaptor ligation, index addition, and library enrichment with limited cycle PCR. Pooled RNA libraries were sequenced using the Illumina HiSeq 2500 system in High Output 2x150 paired-end configuration generating on average 20 million reads per biologic replicate. Data was analyzed by Rosalind (<https://rosalind.onramp.bio/>), with a HyperScale architecture developed by OnRamp BioInformatics, Inc. (San Diego, CA). Reads were trimmed using cutadapt (http://dx.doi.org/10.14806/ej.17.1.200). Quality scores were assessed using FastQC [12]. Reads were aligned to the Homo sapiens genome build hg19 using STAR [13]. Individual sample reads were quantified using HTseq [14] and normalized via Relative Log Expression (RLE) using DESeq2 R library [15]. Read Distribution percentages, violin plots, identity heat-maps, and sample MDS plots were generated as part of the QC step using RSeQC [16]. DEseq2 was also used to calculate fold changes and p-values. Gene clustering for the heatmap of differentially expressed (DE) genes was done using the PAM (Partitioning Around Medoids) method using the fpc R library (Hennig, C. Cran-package fpc. https://cran.rproject.org/web/packages/fpc/index.html). Functional enrichment analysis of pathways, gene ontology, domain structure and other ontologies was performed using HOMER [17]. Additional gene enrichment analyses were performed on unseparated DE genesets by Advaita ([http://www.advaitabio.com/ipathwayguide)](http://www.advaitabio.com/ipathwayguide%29) [18] and on separate upregulated and downregulated genesets (top 2000 DE genes) using ENRICHR [19, 20].

**Statistical analysis**

GraphPad Prism version 7 for Windows (GraphPad Software, San Diego, CA) was used to perform data analyses, variation estimation and validation of test assumptions. The differences between experimental groups in tumor volume, quantification of immunohistochemical analysis were performed with longitudinal data analysis method, independent t-tests, or ANOVA.

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