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Author manuscript

JAMA Oncol. Author manuscript; available in PMC 2015 September 02.

Published in final edited form as:

JAMA Oncol. 2015 May 1; 1(2): 238–244. doi:10.1001/jamaoncol.2015.34.

## Genomic Correlate of Exceptional Erlotinib Response in Head and Neck Squamous Cell Carcinoma

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**Role of the Funder/Sponsor:** The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Disclaimer:** This work does not represent the views of the US government or the Department of Veterans Affairs.

**Additional Contributions:** We appreciate the patient's participation in this study. In addition, we thank Lauren Ambrogio, BS, Broad Institute of Massachusetts Institute of Technology and Harvard University, for project management contributions. She was not compensated for her contribution to the study.

**Author Contributions:** Dr Grandis had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Van Allen, Lui, Egloff, Garraway, and Grandis contributed equally to this work.

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**Obtained funding:** Golub, Mills, Garraway, Grandis.

**Administrative, technical, or material support:** Egloff, Li, Johnson, Duvvuri, Zeng, Wang, Chiosea, Sougnez, Du, Johnston, Mills, Grandis.

**Study supervision:** Van Allen, Egloff, Johnson, Carter, Golub, Mills, Garraway, Grandis.

**Conflict of Interest Disclosures:** Dr Garraway is a consultant and equity holder in Foundation Medicine. Dr Grandis receives research support from Novartis and Bristol-Myers Squibb. No other disclosures are reported.

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## Abstract

**IMPORTANCE**—Randomized clinical trials demonstrate no benefit for epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in unselected patients with head and neck squamous cell carcinoma (HNSCC). However, a patient with stage IVA HNSCC received 13 days of neoadjuvant erlotinib and experienced a near-complete histologic response.

**OBJECTIVE**—To determine a mechanism of exceptional response to erlotinib therapy in HNSCC.

**DESIGN, SETTING, AND PARTICIPANTS**—Single patient with locally advanced HNSCC who received erlotinib monotherapy in a window-of-opportunity clinical trial (patients scheduled to undergo primary cancer surgery are treated briefly with an investigational agent). Whole-exome sequencing of pretreatment tumor and germline patient samples was performed at a quaternary care academic medical center, and a candidate somatic variant was experimentally investigated for mediating erlotinib response.

**INTERVENTION**—A brief course of erlotinib monotherapy followed by surgical resection.

**MAIN OUTCOMES AND MEASURES**—Identification of pretreatment tumor somatic alterations that may contribute to the exceptional response to erlotinib. Hypotheses were formulated regarding enhanced erlotinib response in preclinical models harboring the patient tumor somatic variant *MAPK1* E322K following the identification of tumor somatic variants.

**RESULTS**—No *EGFR* alterations were observed in the pretreatment tumor DNA. Paradoxically, the tumor harbored an activating *MAPK1* E322K mutation (allelic fraction 0.13), which predicts ERK activation and erlotinib resistance in *EGFR*-mutant lung cancer. The HNSCC cells with *MAPK1* E322K exhibited enhanced EGFR phosphorylation and erlotinib sensitivity compared with wild-type *MAPK1* cells.

**CONCLUSIONS AND RELEVANCE**—Selective erlotinib use in HNSCC may be informed by precision oncology approaches.

The discovery of activating mutations in the epidermal growth factor receptor (EGFR) gene accelerated the clinical deployment of small-molecule tyrosine kinase inhibitors (TKIs) that effectively target the altered protein,<sup>1</sup> yielding clinical benefit in many patients with *EGFR*-mutant lung adenocarcinoma.<sup>2</sup> In unselected patients with head and neck squamous cell carcinoma (HNSCC), phase 1/2 trials suggested that EGFR TKIs might be clinically active.<sup>3–7</sup> However, randomized phase 2 and 3 trials of EGFR TKIs for locally advanced or recurrent and/or metastatic HNSCC failed to show clinical benefit when added to standard-of-care regimens in unselected populations.<sup>8,9</sup>

Complete responses to EGFR TKIs in solid tumors are extremely rare, poorly understood,<sup>10</sup> and have been observed in *EGFR* wild-type settings.<sup>11</sup> Genomic correlates of extraordinary response to targeted therapeutics have been demonstrated in other contexts,<sup>12–16</sup> raising the possibility that a rare extreme response to erlotinib hydrochloride may result from somatic alterations in a patient's tumor.

A man with locally advanced HNSCC received neoadjuvant erlotinib for 13 days in a window-of-opportunity clinical trial in which patients scheduled to undergo primary cancer surgery were treated briefly with an investigational agent. Unexpectedly, this patient experienced a near-complete histologic response without recurrence more than 2 years after therapy. Whole-exome sequencing of his pretreatment tumor and germline was performed to investigate molecular profiles permissive of this response.

## Methods

### Study Oversight

The patient provided written informed consent for an institutional review board–approved protocol to perform genomic profiling on tumor and germline DNA.

### Pathologic Analysis and Sequencing

Tumor samples from pretreatment and surgical specimens were reviewed by an HNSCC pathologist (S.C.). Clinical human papillomavirus in situ hybridization testing detecting types 6, 11, 16, 18, 30, 31, 33, 35, 45, 51, and 52 was performed. DNA was extracted from tumor and matched germline, followed by whole-exome sequencing and analysis (eMethods in the Supplement).<sup>18</sup>

### Experimental Analysis

*MAPK1*E322K (HSC-6), wild-type *MAPK1*(CAL-33), and *MAPK1*-deleted (FaDu) HNSCC cells were identified using published resources.<sup>19,20</sup> *MAPK1*-deleted cells were transfected with vector, wild-type *MAPK1* or *MAPK1* E322K expression constructs. Western blotting was performed for EGFR and MAPK pathway members as described previously.<sup>21</sup> Viability and senescence following erlotinib treatment were evaluated in engineered FaDu cells and in HSC-6 cells transfected with MAPK1-targeting or control small interfering RNA (eMethods in the Supplement).

#### At a Glance

- The presence of *MAPK1* E322K predicts resistance to erlotinib therapy in preclinical models but was identified in the tumor of an extraordinary responder.
- Engineered *MAPK1* E322K cells exhibit enhanced erlotinib sensitivity compared with *MAPK1* wild-type cells.
- *MAPK1* E322K induces EGFR activation in head and neck squamous cell carcinoma (HNSCC) in vitro models.
- *MAPK1* E322K is present at low frequencies in HNSCC and cervical cancers.

## Report of a Case

A 32-year-old man presented with a painful lesion on the right side of the oral tongue. The patient drank 6 beers daily and had a 28.5 pack-year smoking history. Biopsy of the lesion revealed invasive squamous cell carcinoma (Figure 1B and C). The tissue was negative for human papillomavirus and p16. Following biopsy, the primary ventral tongue tumor measured 1.9 cm in diameter. Physical examination was notable for palpable right cervical adenopathy (level Ib). A contrast-enhanced computed tomographic scan showed soft-tissue asymmetry of the right side of the tongue, bilateral lymphadenopathy, and no evidence of distant metastatic disease. The patient's disease was clinically staged as T1N2cM0 oral cavity squamous cell carcinoma (stage IVA).

The patient was enrolled in a randomized, placebo-controlled window-of-opportunity clinical trial studying blockade of EGFR and/or Src pathways in HNSCC (NCT00779389). He was randomized to receive erlotinib monotherapy at 150 mg daily for 13 days (Figure 1A). On day 8, the patient developed a facial rash, which has been associated with erlotinib response.<sup>17</sup> On day 14, the patient underwent right partial glossectomy and bilateral modified neck dissection (levels IA-IV). Clinically, the 1.9-cm primary tongue tumor had resolved. Histologic evaluation revealed 2 residual foci (approximately 2 mm each) of invasive, moderately differentiated squamous cell carcinoma within the tongue resection specimen (Figure 1D and E). There was no evidence of lymph node metastasis (0 of 36 nodes in the right neck; 0 of 36 nodes in the left neck). The treated pathologic stage was ypT1N0. The patient received no adjuvant therapy and had no evidence of disease recurrence 24 months postoperatively.

## Results

### Genetic Analysis of the Pretreatment Tumor

The mean target coverage across the exome was 85X in the tumor sample and 183X in the germline sample. There were 39 missense mutations, 3 nonsense mutations, and 2 frame shift deletions, for a nonsilent mutation rate of 2.00 mutations/Mb (eTables 1 and 2 in the Supplement). No somatic mutations, short insertions or deletions, or copy number alterations were observed in *EGFR* (Figure 2A and eTables 3 and 4 in the Supplement), *PTEN*, *PIK3CA*, or *HRAS*.<sup>22–24</sup> Heuristic analysis identified somatic alterations in 4 clinically or biologically relevant cancer genes (Figure 2B): *TP53*,<sup>25,26</sup> *ABL2*, *ARID1A*, and *MAPK1*.

The *MAPK1* alteration (allelic fraction, 0.13; median, 0.15) was particularly unexpected in this patient. *MAPK1* codes for ERK2, a mitogen-activated signaling (MAPK) pathway member downstream of Raf and MEK (Figure 2C), and the E322 locus is highly conserved across species (Figure 2D).<sup>27</sup> The E322K mutation occurs in approximately 1% of HNSCC<sup>28,29</sup> and 8% of cervical squamous cell carcinomas.<sup>30</sup> *MAPK1* E322K causes constitutive activation of ERK2.<sup>20,31</sup> *MAPK1* amplification, which may also activate ERK signaling, confers EGFR TKI resistance in lung adenocarcinoma pre-clinical models.<sup>32</sup> Thus, the presence of *MAPK1* E322K would be predicted to result in ERK signaling

activation and intrinsic resistance, rather than exquisite sensitivity, to EGFR-directed therapies.

### **MAPK1 E322K Effects on EGFR Activation and Erlotinib Sensitivity**

To explore the possibility that the *MAPK1* E322K mutation may have paradoxically conferred exquisite dependency on aberrant EGFR signaling in this patient, wild-type *MAPK1* (CAL-33) and *MAPK1* E322K (HSC-6) HNSCC cells were analyzed for relative EGFR protein and pathway activation (Figure 3A). The HNSCC cells harboring endogenous *MAPK1* E322K expressed higher basal levels of PY1068 EGFR, a surrogate for activated EGFR, and downstream AKT signaling compared with wild-type *MAPK1* cells.

Because CAL-33 and HSC-6 cells have additional non-overlapping alterations, we also engineered FaDu cells, which have a preexisting heterozygous *MAPK1* deletion and are wild type for *PIK3CA*, *PTEN*, *NRAS*, *HRAS*, and *AKT*,<sup>19</sup> to express vector, wild-type *MAPK1* or the *MAPK1*-E322K mutant. The resulting engineered *MAPK1* E322K cells expressed higher basal levels of PY1068 EGFR (Figure 3B) and demonstrated significantly increased senescence (Figure 3C and eFigure 1 in the Supplement) and significantly increased cell death (eFigure 2 in the Supplement) following erlotinib treatment compared with cells expressing wild-type *MAPK1* or vector control cells. Finally, knockdown of ERK2 protein levels in *MAPK1* E322K cells (HSC-6) resulted in reduced cell death following erlotinib treatment compared with parental and nontargeting small interfering RNA transfected cells (eFigure 3 in the Supplement). In aggregate, these data are consistent with the clinical observation that the presence of *MAPK1* E322K can contribute to erlotinib sensitivity in HNSCC.

## **Discussion**

We identified a patient with stage IVA HNSCC who experienced an exceptional response to a 13-day course of neoadjuvant erlotinib. This response occurred in the context of an activating somatic *MAPK1* E322K mutation and thus was particularly noteworthy given that *MAPK1* amplification or activation predicts erlotinib resistance (rather than sensitivity) in preclinical cancer models. We then demonstrated that EGFR and downstream pathway members show increased activation in HNSCC cells harboring *MAPK1* E322K compared with wild-type *MAPK1* cells. We speculate that EGFR dependency may have resulted from an activated Erk-mediated increase in EGFR ligand production in this patient's tumor. Increased levels of amphiregulin as a result of Erk activation have been reported for bronchial epithelial cells,<sup>33</sup> and high levels of amphiregulin have been associated with response to EGFR TKIs.<sup>34</sup>

Although only observed in a single patient, this finding may have important clinical implications. This type of genomic event would not occur at sufficient frequency to influence the results of erlotinib-oriented phase 2/3 studies in unselected patients with HNSCC. Instead, this case highlights the potential contribution of the “long tail” of clinically relevant cancer genes that may prove actionable in some patients.<sup>18</sup>

This study also demonstrates how different activating alterations in the same gene may lead to clinically distinct responses to the same therapy; the effect of 2 different ERK-activating mechanisms (point mutation or amplification) may not be clinically equivalent. This result indicates the promise of more expansive precision oncology efforts linking genomics with clinically annotated cases, so that phenotype-to-genotype relationships can be rapidly identified and studied. Toward this end, it is unknown whether this *MAPK1* E322K mutation may contribute to similar sensitivity to erlotinib therapy in other tumor types (eg, cervical cancer<sup>30</sup>), or with antibody-mediated EGFR inhibition (eg, cetuximab). This will need to be assessed in additional studies.

There are limitations to this study. First, whereas the degree of response to brief erlotinib therapy was impressive, the relative contributions of erlotinib or surgery for the duration of response cannot be definitively determined. Furthermore, whereas forced overexpression of *MAPK1* E322K did increase erlotinib sensitivity in *MAPK1*-deleted HNSCC cells, the *MAPK1* E332K mutation's effect on erlotinib sensitivity was modest. The preclinical models examined here were not derived from this patient's tumor; thus, they may have arisen through different carcinogenic paths or harbor additional alterations that confound the study of erlotinib sensitivity. Conversely, the patient's exceptional response may have been enhanced by other somatic alterations. Importantly, although experimental studies did not fully phenocopy the exquisite erlotinib sensitivity of the patient tumor, the results tracked with the patient's response and countered the expectation that *MAPK1* E332K would confer erlotinib resistance.

## Conclusions

Broadly, these results support the notion that outlier genomics (eg, the National Cancer Institute's Exceptional Responders Initiative) may yield unexpected insights into cancer biology and clinical management for both approved and investigational therapies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Funding/Support:** The study was supported by the American Cancer Society (Drs Van Allen and Grandis), the Patricia L. Knebel Fund of the Pittsburgh Foundation (Dr Lui), the National Cancer Institute K07 CA137140 (Dr Egloff), the Sheikh Khalifa bin Zayed Al Nahyan Institute for Personalized Cancer Therapy (Drs Zhang and Mills), the Slim Initiative for Genomic Medicine, a joint US-Mexico project funded by the Carlos Slim Health Institute (Drs Golub and Garraway), the National Cancer Institute Head and Neck Cancer Specialized Program of Research Excellence P50 CA097190 (Dr Grandis), OSI Pharmaceuticals (Dr Grandis), and Bristol-Myers Squibb (Dr Grandis). This work was supported in part by the US Department of Veterans Affairs Career Development Award, Biomedical Laboratory Research and Development (Dr Duvvuri).

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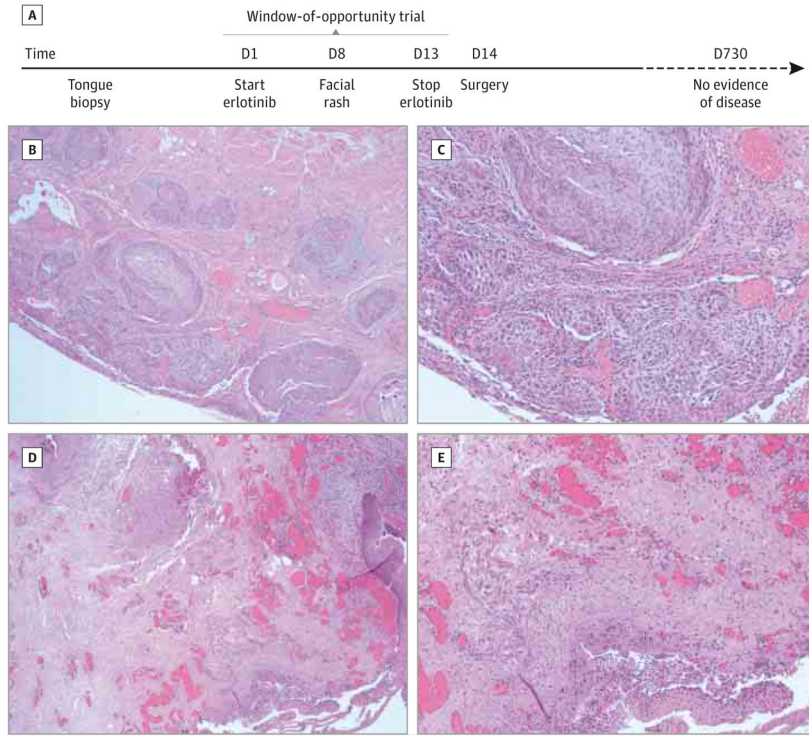
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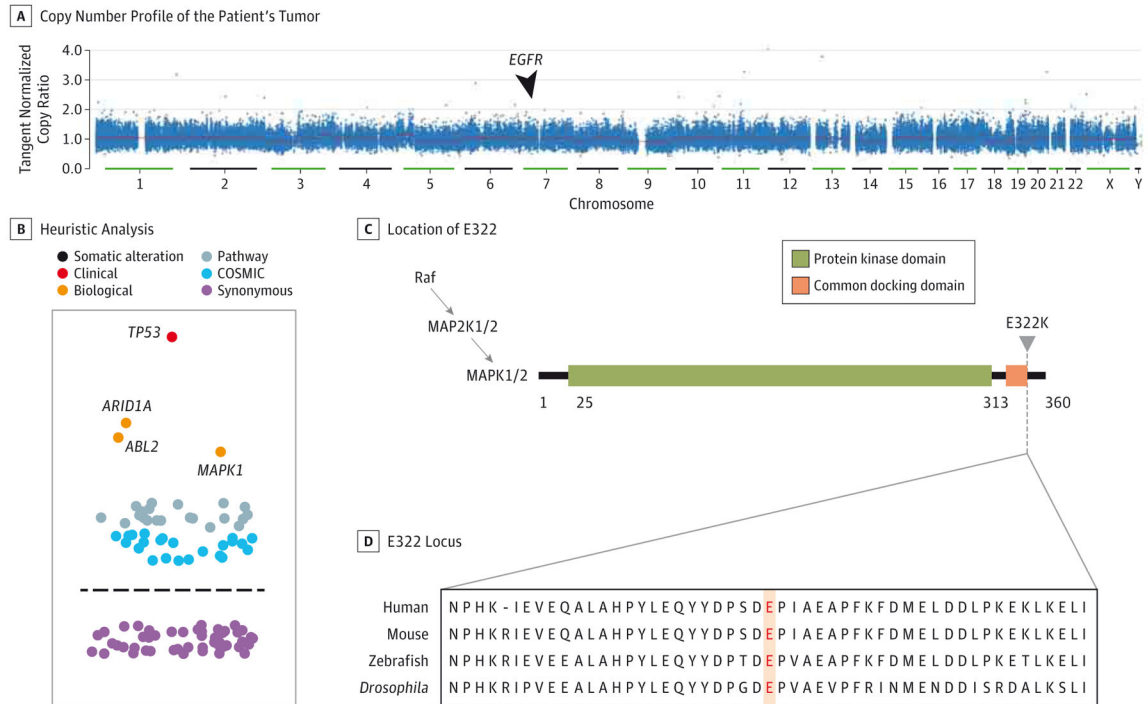
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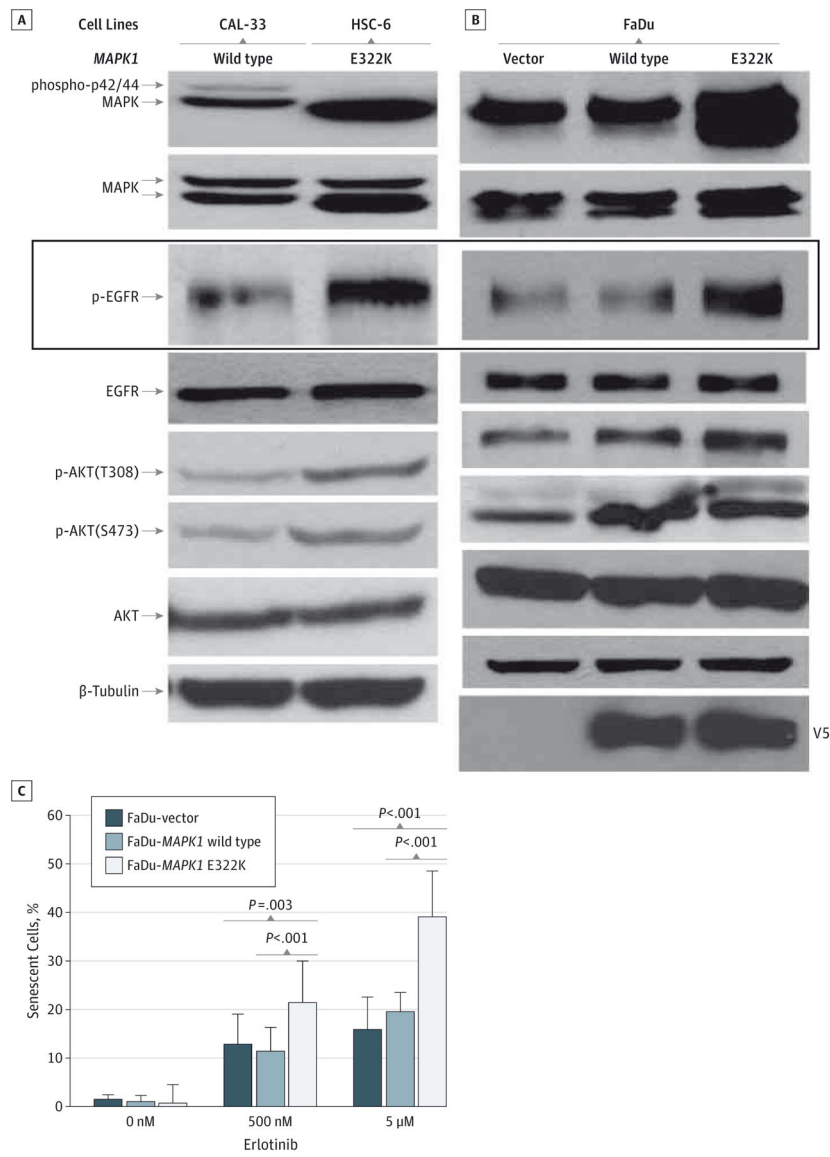
**Figure 1. Clinical Course and Histologic Findings**

A, Time course for the patient’s clinical experience, highlighting the number of days receiving erlotinib and the time elapsed since surgery. D indicates day. B and C, Representative histologic analysis images of the pretreatment tumor biopsy confirm squamous cell carcinoma (hematoxylin-eosin; B, original magnification  $\times 40$ ; C, original magnification  $\times 100$ ). D and E, representative histology images from surgical specimens taken after 13 days of erlotinib therapy (hematoxylin-eosin; D, original magnification  $\times 40$ ; E, original magnification  $\times 100$ ).



**Figure 2. Whole-Exome Sequencing of the Pretreatment Tumor**

A, There is no evidence of *EGFR* amplification. B, Heuristic analysis of the somatic mutations, short insertions and deletions, and copy number alterations across the exome identifies 4 mutations for additional evaluation: *TP53*, *ARID1A*, *ABL2*, and *MAPK1*. The horizontal line denotes the separation between nonsynonymous and synonymous variants, and COSMIC indicates the Catalogue of Somatic Mutations in Cancer database. C, The location of E322 is near the terminal end of the MAPK1 protein, in the common docking domain. Numbers indicate the color-coded regions of the protein. D, The E322 locus is highly conserved across species.



**Figure 3. MAPK1 E322K Mutations and EGFR Signaling**

A, HSC-6 cells that harbor an endogenous *MAPK1* E322K mutation express higher basal levels of phospho-p42/44 MAPK, p-EGFR(Y1068), and pAKT(S473) when compared with CAL-33 cells that endogenously express *MAPK1* wild type. B, The head and neck squamous cell carcinoma cells with deleted *MAPK1* (FaDu) were engineered to express increased levels of mutant E322K and demonstrate upregulation of phospho-p42/44 MAPK, p-EGFR(Y1068), and pAKT(S473) levels compared with vector-control transfectants. Similar results were observed at least 3 times. C, FaDu-*MAPK1* E322K cells demonstrated significantly increased senescence compared with FaDu-vector and FaDu-wild-type *MAPK1* following 48-hour treatment with erlotinib.  $\beta$ -Galactosidase activity at pH 6 was detected in senescent cells by means of light microscopy (original magnification  $\times 100$ ) following staining using the senescence staining kit (Cell Signaling Technology). Number of senescent cells and total number of cells per field were analyzed for at least 5 fields for each cell type

and treatment condition. Mean (SD) percent of senescent cells from a representative of 3 independent experiments is presented.

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