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High Expression of the RET Receptor Tyrosine Kinase and Its Ligand GDNF Identifies a High-Risk Subset of Estrogen Receptor Positive Breast Cancer

Rasha T. Kakati, MD¹, Hyunsoo Kim, PhD¹, Austin Whitman, MSc¹, Philip M. Spanheimer, MD FACS^{1,2}

¹Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

²Department of Surgery, University of North Carolina, Chapel Hill, NC

Abstract

Purpose: Resistance to endocrine therapy is the primary cause of treatment failure and death in patients with ER-positive (ER+)/luminal breast cancer. Expression and activation of the RET receptor tyrosine kinase may be driving poor outcomes. We aim to identify high-risk patients and druggable pathways for biomarker-based clinical trials.

Methods: We obtained batch-normalized mRNA expression data from Breast Invasive Carcinoma - The Cancer Genome Atlas, PanCancer Atlas (BRCA-TCGA). To determine clinically significant cutoffs for RET expression, patients were grouped at different thresholds for Kaplan-Meier plotting. Differential gene expression (DGE) analysis and enrichment for gene sets was performed. transcriptomic dataset of antiestrogen-treated ER+ tumors stratified by clinical response was then analyzed.

Results: High RET expression was associated with worse outcomes in patients with ER+ tumors, and stratification was enhanced by incorporating GDNF expression. High RET/GDNF patients had significantly lower overall survival (HR=2.04, p=0.012), progression-free survival (HR=2.87, p<0.001), disease-free survival (HR=2.67, p<0.001), and disease-specific survival (HR=3.53, p<0.001) than all other ER+ patients. High RET/GDNF tumors were enriched for estrogen-independent signaling and targetable pathways including NTRK, PI3K, and KRAS. Tumors with adaptive resistance to endocrine therapy were enriched for gene expression signatures of high RET/GDNF primary tumors.

Conclusion: Expression and activation of the RET receptor tyrosine kinase may be driving poor outcomes in some patients with ER+ breast cancer. ER+ patients above the 75th percentile may benefit from clinical trials with tyrosine kinase inhibitors.

Correspondence should be addressed to: Philip M. Spanheimer MD FACS, Assistant Professor, Department of Surgery, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, 170 Manning Drive, Suite 1149, Chapel Hill, NC 27599-7213 USA, Phone: 919-966-5221; Fax: 919-966-8806, Philip.Spanheimer@med.unc.edu.

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Keywords

Breast cancer; RET; GDNF; MAPK; estrogen receptor signaling

INTRODUCTION

Breast cancer is the most prevalent cancer and the second leading cause of cancer-related death in women globally[1,2]. Molecular subtypes have been identified with distinct transcriptomic profiles, treatment responses, and outcomes[3]. Approximately 80% of breast tumors are categorized within the luminal subtypes characterized by expression of the estrogen (ER) and progesterone receptors (PR)[3,4]. ER+/Luminal tumors have a relatively favorable prognosis compared to other subtypes[3,4]. However, roughly 30% of patients with ER+ tumors are innately resistant or develop resistance to endocrine therapy[5]. Endocrine resistance is a significant challenge in the treatment of ER+ breast cancer and various studies have been conducted to understand underlying mechanisms of resistance[4,6,7]. Multiple signaling pathways and modulators have been identified contributing to resistance including gain of function mutations in *ESR1* encoding the estrogen receptor, dysregulation of MAPK signaling, and upregulation of receptor tyrosine kinases (RTKs)[1,6,8]. Nevertheless, specific drivers of endocrine resistance remain unidentified for the majority of ER+ breast cancer patients[6].

Several previous studies have established a connection between the RET RTK and response to endocrine therapy. RET activity results in downstream phosphorylation of ER when stimulated by its ligand glial cell derived neurotrophic factor (GDNF)[8,9] which leads to estrogen independent activation of estrogen response elements. Additionally, our prior work has demonstrated that RET is a significant modulator of ERK/MAPK activation and that genetic or pharmacologic inhibition of RET can enhance sensitivity to tamoxifen in ER+ breast cancer cell lines[9,10]. We hypothesize that a high expression of RET in human ER+ tumors is associated with poor outcomes and that RET activity may differentiate a distinct biological subset of ER+ tumors with specific gene expression patterns. Unique features of RET active tumors will identify biomarkers of patients at high risk for recurrence for future RET inhibitor trials.

METHODS

cBioPortal was used to access Breast Invasive Carcinoma The Cancer Genome Atlas, PanCancer Atlas (BRCA-TCGA) clinical and genomic data[11,12]. Gene expression data was obtained using batch-normalized mRNA expression RSEM data. Clinical and sample information was compiled from the clinical attributes and sample attributes files. Tumors were assigned PAM50 intrinsic subtype using a normalized centroid predictor[13]. 1,081 breast cancer patients with RSEM and clinical data were identified from BRCA-TCGA. Statistical analysis was performed using RStudio.

To analyze the distribution of gene expression across PAM50 subtypes and ER status, boxplots were generated using ggplot2, ggbreak, ggpubr, and scales R packages, and statistical significance was determined by student's t-tests between groups as well as log2

fold changes (LogFC) in specific gene expression between groups of interest. Patients with missing data or indeterminate ER status were omitted from the analysis. *GDNF* expression was identified using established aliases[14,15].

To determine clinically significant cutoffs for differential gene expression analysis (DGE), patients were grouped and Kaplan-Meier (KM) plots were plotted for overall survival (OS), progression-free survival (PFS), disease-free survival (DFS) and disease-specific survival (DSS) using the survival[16] and survminer[17] packages. We systematically investigated various thresholds to determine an optimal *RET* expression threshold for high or low-expression groups.

For DGE, genes with less than 10 counts across all patients were omitted before analysis of RSEM data using the DESeq2[18] R package. Significant differentially expressed genes were grouped by regulation status using a LogFC cutoff of 1 for upregulation and -1 for downregulation, along with a false discovery rate (FDR) < 0.05. Genes with FDR < 0.05 but not meeting the upregulation or downregulation cutoff were indicated as increased or reduced in expression. Volcano plots of all genes were plotted using the EnhancedVolcano package and a heatmap plotted using the ComplexHeatmap[19] package. Enrichment of gene sets was then investigated through hypergeometric analysis of upregulated or downregulated elements using clusterProfiler[20]. Enrichment analysis was then performed using MSigDB[21] sets and gene ratios were computed as the number of genes enriched relative to the total number of genes from the specified set. Enriched sets were grouped by categories of interest and plotted using ggplot2[22]. Specific genes from enriched gene sets were then investigated through a heatmap using the ggplot2[22] package. Notable, enriched pathways were then analyzed through networks to map LogFC values of interacting genes using Cytoscape[23].

To determine changes in *RET* and associated gene expression with endocrine resistance, we obtained gene expression data from Xia et al.[7]. In that study, mRNA sequencing was performed from tumor samples while patients were taking endocrine therapy and annotated with clinical response to therapy (i.e. sensitivity and resistance). Gene counts were normalized to the upper quartile using NOISeq[24] and counts were log2 transformed. Scores for gene sets of interest were computed across each sample using singscore[25]. Boxplots were generated for *RET* and *GDNF* expression and gene set scores from TCGA analyses across resistant and sensitive samples. Scatter plots of *RET* and *GDNF* expression levels and bar plots of response type by *RET* and *GDNF* co-expression status were also generated using ggplot2[22].

AVAILABILITY OF DATA AND MATERIALS

The datasets and codes supporting the conclusions of this article are available in the GitHub repository, www.github.com/rashatk/HighRETGDNF.

RESULTS

RET Expression Across the Landscape of Human Breast Cancer

We used the TCGA transcriptional data to investigate expression of *RET* across intrinsic (PAM50) subtypes to determine how *RET* expression is associated with phenotypes. *RET* expression was enriched in luminal and HER2-enriched subtypes compared to basal, claudin-low, and normal-like ($p < 0.001$) (Figure 1a). Luminal B tumors had the highest median *RET* expression, (LumB vs Basal LogFC=4.20, $p < 0.001$). Expression of *RET* in Luminal A tumors was significantly lower than Luminal B (LumA vs LumB LogFC=-0.44, $p = 0.005$) (Figure 1a). *RET* expression strongly correlated with clinical ER status, with the 794 ER+ patients having significantly higher *RET* expression compared to ER- (LogFC=2.90, $p < 0.001$) (Figure 1b).

RET Expression and Outcomes in ER+ Breast Tumors

ER positivity is widely clinically available and used for treatment decisions, so we analyzed the impact of *RET* expression on cancer outcomes in patients with ER+ breast cancer. We employed a systematic analysis based on the 10th, 25th, 50th, 75th and 90th percentiles to determine a biologically relevant threshold of *RET* expression that could function as a biomarker of worse outcome. *RET* expression above the 75th percentile (high *RET*) demonstrated the most robust stratification of outcomes. Kaplan-Meier (KM) plots demonstrated significantly lower progression-free survival (PFS) (HR=1.66, $p = 0.018$), disease-free survival (DSS) (HR=1.77, $p = 0.049$), and disease-specific survival (DSS) (HR=2.13, $p = 0.006$) for high *RET* patients (Figure 2a). There was additionally a trend towards worse overall survival (OS) in high *RET* patients, (HR=1.38, $p = 0.13$) (Figure 2a).

Impact of RET-GDNF Co-expression on Survival in ER+ Patients

Because *RET* expression may not be sufficient for activity of the *RET* receptor and downstream signaling pathways, we investigated co-expression of *RET* with its activating ligand *GDNF*. We utilized the previously identified 75th percentile threshold for *RET* expression and stratified tumors based on both *RET* and *GDNF* expression above the 75th percentile (high *RET*/*GDNF*) compared to all other ER+ tumors. Fifty-six of 739 ER+ tumors (7.6%) were in the high *RET*/*GDNF* group. KM plots demonstrated that high *RET*/*GDNF* patients had significantly lower OS (HR=2.04, $p = 0.012$), PFS (HR=2.87, $p < 0.001$), DFS (HR=2.67, $p < 0.001$), and DSS (HR=3.53, $p < 0.001$) than all other ER+ patients (Figure 2b). Co-expression of *GDNF* and *RET* demonstrated more robust stratification of outcomes compared to *RET* expression alone.

We compared features of patients with high *RET*/*GDNF* to all other ER+ patients (Table 1). High *RET*/*GDNF* tumors were more common among white women ($p = 0.019$) and tumors with ductal histology ($p < 0.001$). Notably, there were no significant differences in age, stage, or PAM50 subtype, which imply that unique biology, rather than previously identified prognostic factors are driving poor outcomes.

Gene Expression Patterns of High RET & GDNF ER+ Breast Tumors

Differential gene expression (DGE) analysis comparing high RET/GDNF tumors to the remaining ER+ tumors revealed 195 significantly upregulated and 945 downregulated genes (Figure 3a). The top 40 upregulated and top 40 downregulated genes were visualized using a heatmap (Figure 3b).

Multiple genes with known roles in estrogen signaling were altered in high RET/GDNF tumors. These include *FGF10* (LogFC=2.124, FDR<0.001), an FGFR2 ligand associated with reduced estrogen dependence, resistance to endocrine therapy, and poor prognosis in ER+ breast cancer.[26]. *KCNK5* which promotes estrogen-dependent proliferation, is reduced (LogFC=-0.978, FDR<0.001)[27,28]. *CCND1*, an indirect transcriptional target of ER driving proliferation and tumor aggression, is increased (LogFC=0.559, FDR=0.004) in high RET/GDNF tumors[29].

We then examined genes associated with endocrine resistance. *MUC2* has been implicated in poor outcomes with aromatase inhibitor therapy and is upregulated (LogFC=3.331, p<0.001) [30]. We also investigated genes established by Wang et al to have a role in tamoxifen resistance including *MAPK1*, *ESR1* and *RANBP2*, which is implicated in JNK/c-JUN signaling[31]. Among high RET/GDNF patients, expression of *MAPK1* (LogFC=0.377, p<0.001), *ESR1* (LogFC=0.642, p=0.001), and *RANBP2* (LogFC=0.46, p<0.001) was increased.

Transcription factors including *NF-κB* and *JAK/STAT* were also altered. *NF-κB* inhibitors such as *IKKB* are implicated in anti-apoptotic signaling; *IKKB* expression is increased (LogFC=0.304, FDR=0.012) and *NF-κB* levels are reduced (LFC=-0.626, FDR<0.001), suggestive of reduced *NF-κB* signaling[32]. Further, *MUC16* is implicated in *JAK/STAT* signaling that leads to c-JUN overexpression[32]. *MUC16* downregulation (LogFC=-2.054, FDR<0.001) and subsequent increase in c-JUN expression (LogFC=-0.532, FDR=0.001) also suggest reduced signaling. Downregulated *NPY5R* (LogFC=-1.171, FDR=0.020), which induces breast tumor apoptosis and reduces *STAT3* activation, may indicate uncontrolled proliferation mediated by overactivation of *STAT3* signaling[33]. *SPHK1* expression is induced by *TNFA* and has been implicated in breast cancer proliferation and metastasis[34]. Reduced *SPHK1* expression may thus result from reduced *TNFA* signaling.

Enriched Signaling Pathways Among ER+ Breast Tumors with High RET & GDNF

To define biologic differences in high RET/GDNF tumors, we analyzed enrichment of key pathway gene sets[21,35]. Gene sets consistent with increased ER signaling were enriched in high RET/GDNF tumors (Figure 4a), as were genes sets of reduced response to estrogen (Figure 4b) and increased antiestrogen resistance (Figure 4c). We compiled a heat plot of notable and significantly altered genes within these sets (Figure 4d), which identified several important genes. *FOXC1* has been shown to repress expression of *ESR1*; and downregulation of *FOXC1* (LogFC=-1.161, FDR<0.001) was seen in high RET/GDNF tumors[36]. *HSD17B1* promotes conversion of estrone into estrogen in the final step of estrogen synthesis[37], so downregulated *HSD17B1* (LogFC=-1.117, FDR<0.001) may indicate divergence towards estrogen-independent ER activity. Similarly,

LEP is implicated in estrogen production and is thus implicated in estrogen-dependent breast cancer growth[38]. Downregulated *LEP* (LogFC=-1.974, FDR<0.001) suggests low estrogen synthesis and estrogen independence. Further, *TRIM29* is a tumor suppressor in ER+ breast cancer that interacts with *ESR1* to reduce binding to estrogen response elements (ERE) that typically results in ER-dependent gene expression[39]. *TRIM29* downregulation (LogFC=-1.469, FDR<0.001) in high RET/GDNF tumors may indicate increased *ESR1* binding to ERE. Additionally, *MYC* is an established direct target of ER and is associated with poor survival, tumor stemness, and aggressive phenotype[40], was enriched (Figure 4e).

Because RET is upstream of ERK/MAPK signaling, we defined expression of genes associated with these pathways in high RET/GDNF ER+ tumors. KRAS and MAPK10 signaling genes were enriched (Figure 4f). DUSP proteins dephosphorylates MAPK proteins leading to reduced MAPK signaling activity[41–43]. Increased expression of *DUSP7* (LogFC=-0.245, p=0.003) and *DUSP4* (LogFC=0.964, FDR<0.001) in high RET/GDNF tumors may activate MAPK. Increased *DCLK1* (LogFC=0.922, FDR<0.001) and *EFR3B* (LogFC=0.816, FDR<0.001) may contribute to increased RAS/ERK activity[44,45]. Expression of PDPK1 which activates AKT is increased (LogFC=0.441, FDR<0.001)[32]. Upstream targets of MAPK and PI3K/AKT signaling were also enriched, including ERBB2 pathway. *TAOK1* (LogFC=1.360, FDR<0.001) and *ERBB4* (LogFC=0.629, FDR=0.004) which activate MAPK were enriched[46,47]. Expression of AREG, an EGFR ligand, is significantly downregulated (LogFC=-1.254, FDR<0.001)[48]. Further, expression of VEGF and VEGFR, which are also upstream regulators of MAPK and AKT signaling, are downregulated[49]. These findings further indicate RET as the driver of KRAS, MAPK and PI3K/AKT signaling in these tumors.

ER and MAPK Networks in High RET/GDNF ER+ Tumors

We used Cytoscape[23] to define interactions in key pathways, including RAS. In this pathway, *GAB1*, *PTPN11* and *RAB5A* expression was increased in high RET/GDNF tumors. *NTRK2* expression was significantly upregulated (LogFC=1.447, FDR<0.001). *NTRK2* has been associated with poor prognosis of invasive breast cancer and represents a druggable target with histology agnostic FDA approval[50–52]. Within PI3K/AKT signaling, *VTN* (LogFC=2.197, FDR<0.001) was upregulated in high RET/GDNF tumors[53]. Genes downstream of the classical estrogen-dependent ER transcriptional activity, such as *TP53*, *FGF2*, and *E2F1*, and the nonclassical estrogen-dependent ER transcriptional activity mediated by transcription factors including *SPI* and *c-JUN* such as *BCL2*, *IGF1*, and *MMPI*, are not significantly altered (Figure 5)[54,55]. However, genes downstream of estrogen-independent ER transcriptional activity are significantly altered, with *RBBP4*, *PTPRF*, and *RAB14* increased in expression[56].

RET Expression in Endocrine Therapy Resistant ER+ Breast Cancer

Because RET reduces sensitivity to endocrine therapy *in vitro*, we hypothesized that RET expression would be enriched in ER+ tumors that are resistant to endocrine therapy. To test this hypothesis, we obtained a transcriptomic dataset from patients with unresectable or metastatic ER+ breast cancer treated with endocrine therapy. Serial biopsies were obtained for whole transcriptome profiling with clinical sensitivity annotation[7].

We compared *RET* and *GDNF* gene expression stratified by endocrine therapy sensitivity. Although *GDNF* levels did not significantly differ among endocrine-resistant tumors relative to sensitive tumors, *RET* gene expression was significantly higher (LogFC=1.025, FDR=0.01) (Figure 6a). *RET* expression was also significantly higher in adaptive resistance samples relative to all sensitive samples (LogFC=1.113, FDR=0.014) (Figure 6b). To directly assess if *RET* and *GDNF* are overexpressed in response to endocrine therapy with emerging resistance, we compared adaptive resistance samples to patient-matched sensitive samples (i.e. sensitive tumors that developed adaptive resistance), which were labeled “patient-matched pre-resistant” (PMP). No differences were observed in *RET* or *GDNF* expression between matched specimens (Figure 6c). Resistant samples demonstrated higher *RET* expression (Figure 6d). Samples were then divided into high RET/GDNF (75th percentile or non-high RET/GDNF. Most sensitive samples were non-high RET/GDNF, whereas the high RET/GDNF samples were enriched for PMP and adaptive resistance samples (Figure 6e).

Gene signatures can be more representative of underlying biology than individual genes, so we characterized the enrichment and depletion of high RET/GDNF gene expression signatures derived from the TCGA analysis. The top 40 upregulated genes and 40 downregulated genes were compiled into gene sets. The upregulated high RET/GDNF signature was not enriched in resistant tumors, but resistant samples were depleted for the downregulated signature (LogFC=-0.037, FDR=0.020) (Figure 6f). Adaptive resistance samples, however, were enriched for the upregulated signature compared to both sensitive (LogFC=0.024, FDR=0.003) and intrinsic resistance (LogFC=0.029, FDR=0.015) samples, as well as significantly lower gene expression for the downregulated gene set relative to sensitive samples (LogFC=-0.035, FDR=0.048) (Figure 6g). Further patient-matched comparison demonstrated significantly higher expression scores of upregulated genes in adaptive resistant samples relative to PMP samples (LogFC=0.032, FDR=0.002), and lower expression of downregulated genes (LogFC=-0.058, FDR=0.002) (Figure 6h). These findings suggest that the development of resistance to endocrine therapy may involve an adaptive response resulting in a gene expression profile characteristic of high RET/GDNF tumors.

DISCUSSION

In this study, we demonstrate a distinct, high-risk subpopulation of ER+ breast cancer characterized by high expression of genes encoding the RET receptor and its activating ligand *GDNF* with poor survival. High RET/GDNF ER+ tumors have a distinct transcriptional profile that underlies important biologic distinctions with other ER+ breast tumors, and could inform treatment strategies for these patients.

We have previously shown that RET inhibition reduces proliferation in ER+ breast cancer cell lines and xenografts[10,57]. However, clinical trials of tyrosine kinase inhibitors in non-HER2 amplified breast cancer have had disappointing results[58–61] which may be due to lack of biomarkers of patients likely to respond. Supporting this, two recent trials of TKI with anti-RET activity were positive by objective response rate in ER+/HER2- patients[62,63]. Biomarker-based selection of patients will be key to identifying patients

most likely to benefit in future clinical trials. The data in this manuscript support the selection of patients above the 75th percentile of both *RET* and *GDNF* expression which identifies patients with poor outcomes and with biological evidence of downstream kinase and estrogen signaling pathways, making this an ideal group for future TKI investigation.

We also identify several key enriched features of high *RET*/*GDNF* tumors that highlight differences in tumor biology and suggest actionable targets in these high-risk patients. High *RET*/*GDNF* drives downstream upregulation of both estrogen-dependent and estrogen-independent ER activity. Interestingly, this appears to be driven by a shift towards estrogen independent ER activity which is associated with increased proliferation, invasion, and metastasis. Because high *RET*/*GDNF* tumors have a shift in ER activity towards estrogen-independent ER activity, ER degraders such as fulvestrant may be preferred over agents such as tamoxifen or aromatase inhibitors that act on estrogen-dependent activation of ER. We have identified multiple druggable targets, including *NTRK2* and *TAOK1* which may demonstrate potential through further investigation to improve outcomes in this population through TKI treatment.

The shift towards ER-independent ER activity may be due to the activation of MAPK pathways downstream of *RET*. MAPK activation has been shown to drive estrogen-independent activation of estrogen response elements in breast cancer cell lines[64], and activating mutations in MPAK pathway can drive endocrine resistance[6,65]. We found enrichment of MAPK activity signatures in high *RET*/*GDNF* ER+ tumors, demonstrating that these pathways are maintained in human breast tumors and may enhance estrogen-independent ER activity (Figure 7). Supporting this, our preoperative window trial using a short treatment of the tyrosine kinase inhibitor vandetanib in primary ER+ tumors results in a reduction in ERK activity[66]. Several inhibitors of ERK/MAPK are currently in clinical trials for breast cancer, and patients with high *RET*/*GDNF* ER+ tumors may be the patient population most likely to benefit from these therapies. Further study is needed to determine the biological mechanisms of *RET* and *GDNF* dependent reprogramming kinase signaling pathways, estrogen response elements, and effects on emerging endocrine resistance.

CONCLUSION

A sub-population of ER+ breast tumors with high *RET*/*GDNF* expression confers poor survival which is associated with MAPK activation and estrogen-independent ER activation. Our analysis identifies the top quartile of *GDNF* and *RET* co-expression as a candidate biomarker for trials of anti-*RET* therapeutics. Additionally, we identify several targetable pathways including *NTRK*, *PI3K*, and *KRAS* that could improve outcomes for these high-risk patients. Further study is needed to understand how *RET* is driving oncogenic pathways in human ER+ tumors and treatment strategies to improve outcomes for patients with *RET*-driven tumors.

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Data availability:

The datasets used and code generated during the current study are available on a public GitHub repository (www.github.com/rashatk/HighRETGDNF). The dataset obtained from Xia et al. can be accessed at doi:[10.1158/1078-0432.CCR-21-3189](https://doi.org/10.1158/1078-0432.CCR-21-3189).

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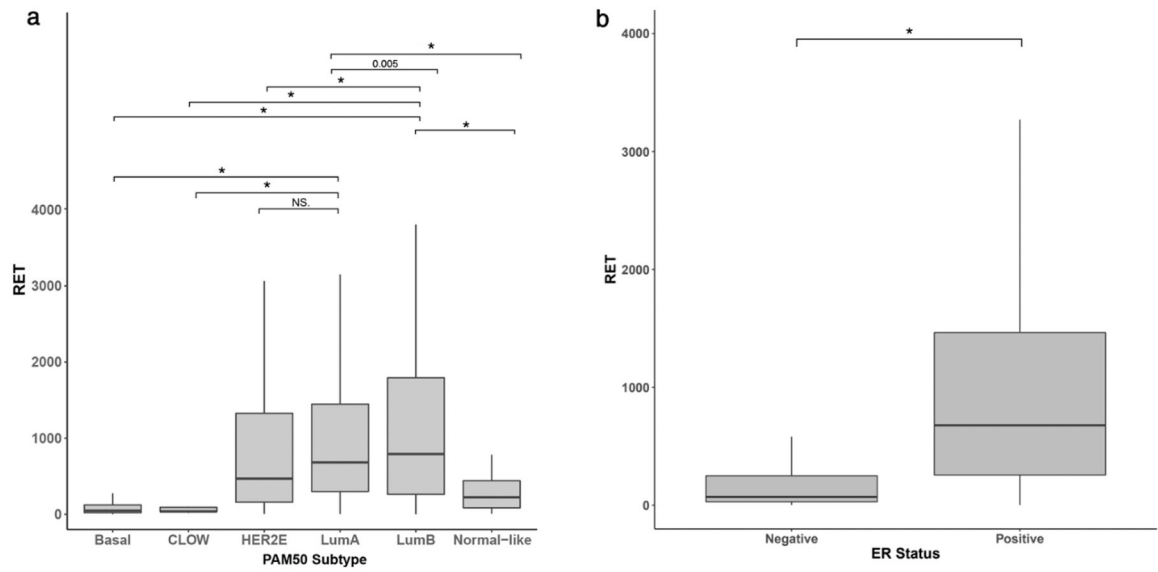


Fig. 1. Distribution of RET gene counts in TCGA human breast tumors stratified by (A) PAM50 subtypes and (B) ER status (NS: Not significant, * $p < 0.001$).

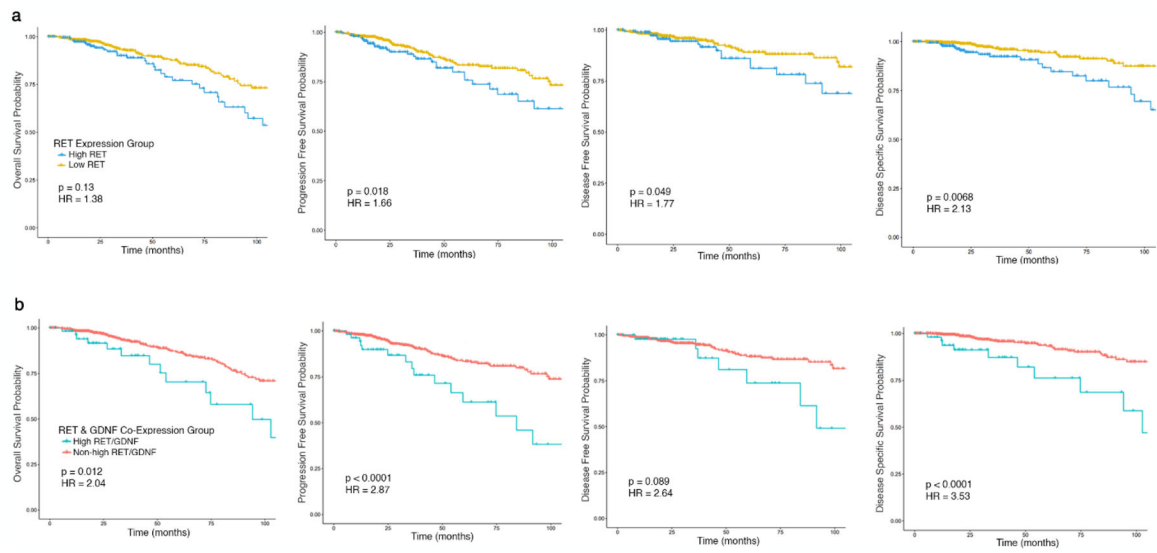


Fig. 2. Kaplan-Meier survival plots comparing ER+ patients (A) above the upper quartile of RET expression (high RET) relative to those below (low RET) or (B) above the upper quartile of both RET and GDNF expression (high RET/GDNF) relative to all others (non-high RET/GDNF) by overall survival, progression-free survival, disease-free survival, and disease-specific survival.

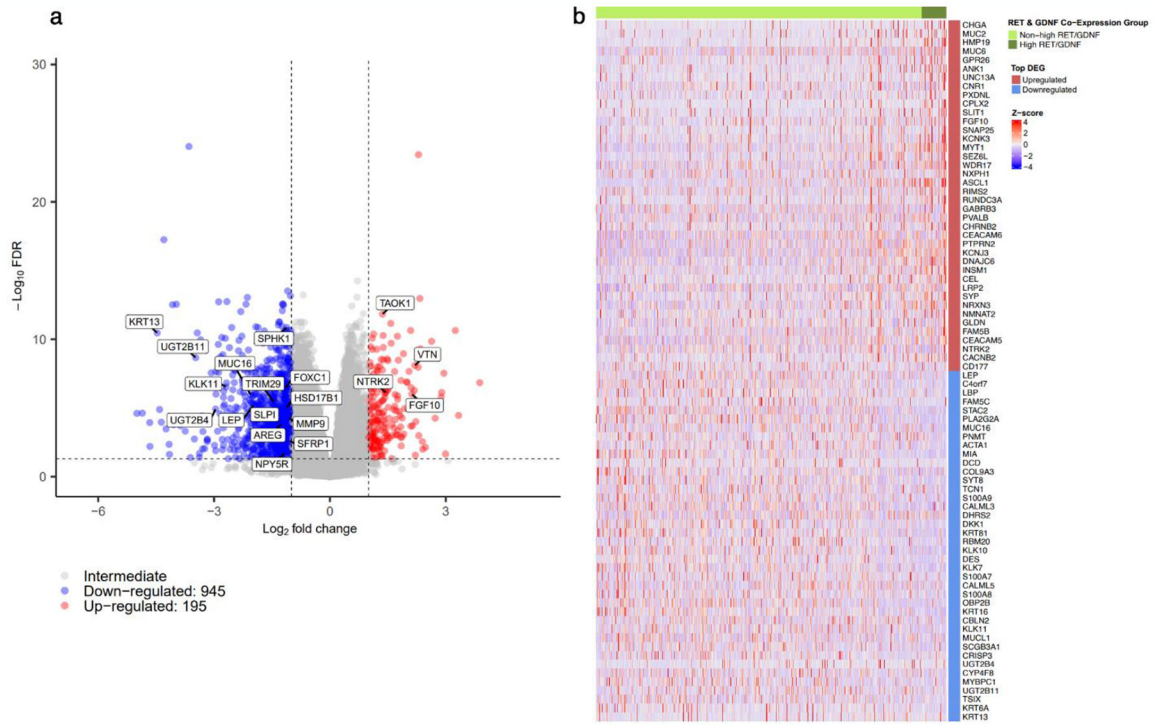


Fig. 3. Differential expression analysis of ER+ patients above the 75th percentile of RET and GDNF expression (high RET/GDNF) relative to all other ER+ patients demonstrated by a (A) volcano plot of all genes with significantly upregulated and downregulated genes having $\text{L2FC} > 1$ and < -1 respectively along with $\text{FDR} < 0.05$ and (B) heatmap of top 40 significant upregulated and downregulated differentially expressed genes defining high RET/GDNF ER+ tumors.

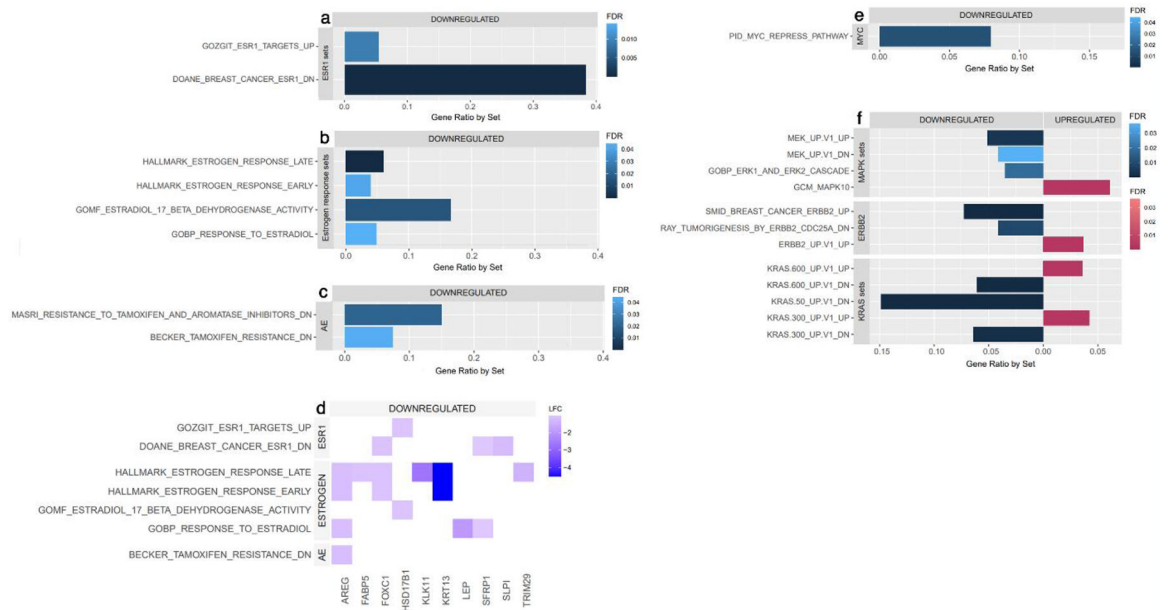


Fig. 4. Enriched gene sets among high RET/GDNF tumors relative to non-high RET/GDNF tumors across (A) ESR1, (B) estrogen, and (C) antiestrogen (AE) signaling sets. (D) Heat plot of notable and significantly regulated genes across enriched estrogen, ESR1 and antiestrogen (AE) signaling sets. Enriched gene sets among high RET/GDNF tumors across (E) MYC and (F) MAPK, ERBB2, and KRAS signaling sets.

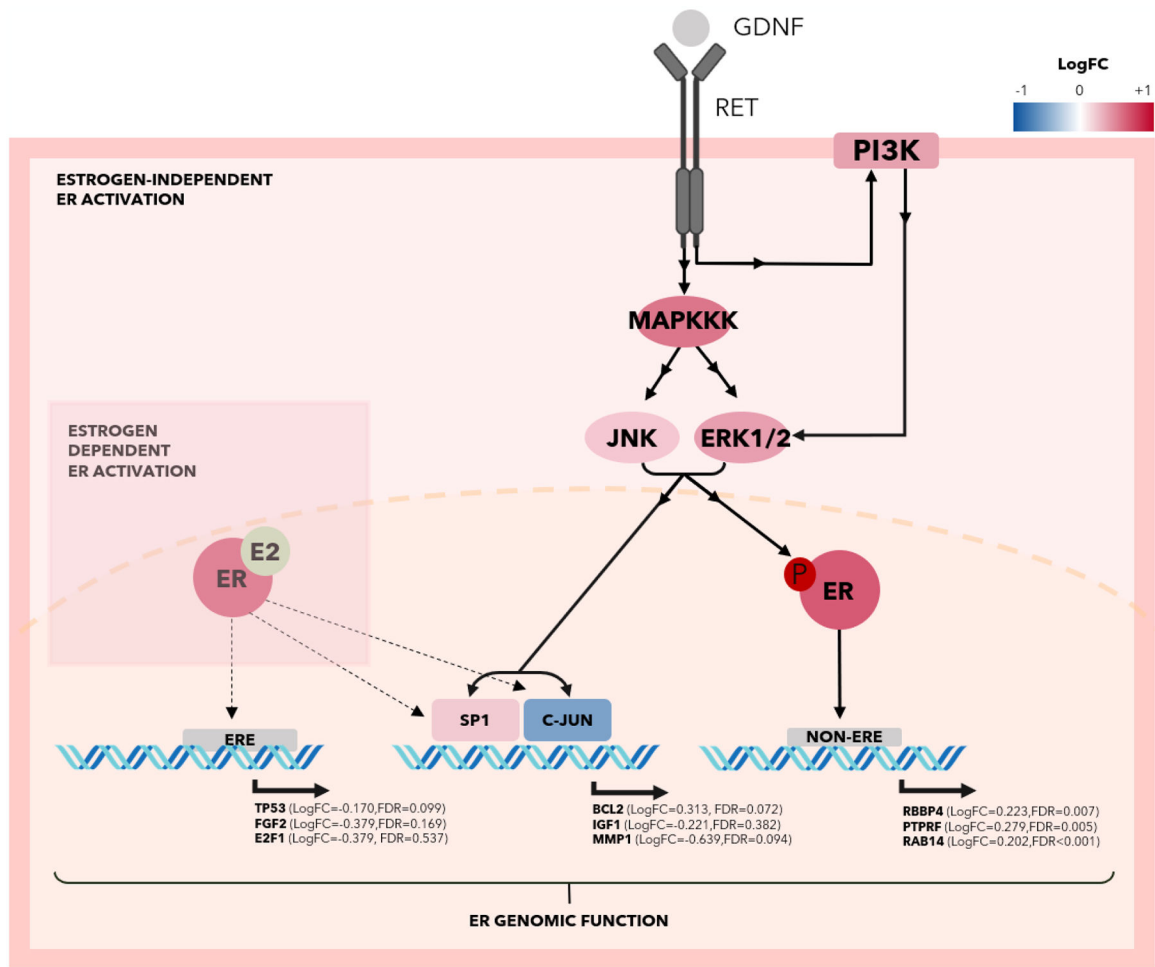


Fig. 5. Graphical representation using Cytoscape of RET activation by GDNF driving activation of MAPK pathway and enriching estrogen-independent ER activity.

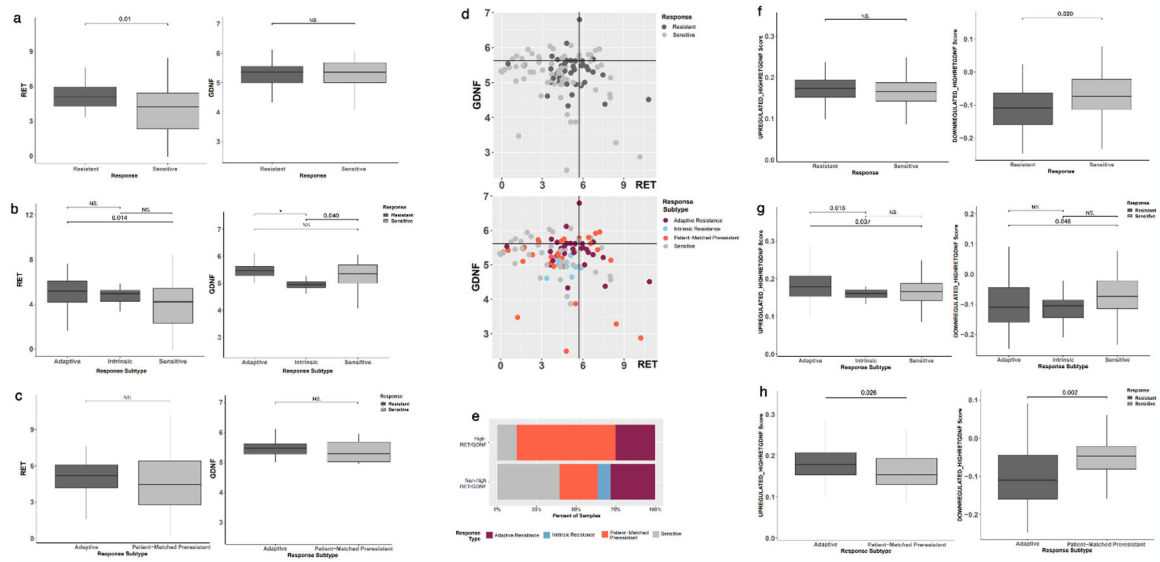


Fig. 6. Expression of RET, GDNF, and signatures of high RET/GDNF profile in a gene expression dataset of endocrine therapy-resistant tumors[7]. **(A)** RET expression is increased in endocrine therapy-resistant tumors compared to sensitive ones with no change in GDNF expression. **(B)** Adaptive-resistance samples have increased RET expression compared to sensitive samples, with no statistically significant difference between intrinsic resistant and sensitive specimens. **(C)** No change in RET or GDNF expression is seen in adaptive-resistance samples and patient-matched resistant (PMP) samples. **(D)** Scatter plot of RET and GDNF expression across all samples by the response to endocrine therapy with cutoffs at the 75th percentile of gene expression. **(E)** Bar plot of response type distribution for samples with high RET/GDNF compared to non-high RET/GDNF samples across all samples. Distribution of enrichment scores for TCGA-derived top 40 upregulated or downregulated gene sets in high RET/GDNF tumors by **(F)** tumor response to endocrine therapy and **(G)** adaptive or intrinsic resistance. **(H)** a comparison between adaptive resistant samples and patient-matched resistant samples demonstrates significant enrichment of upregulated genes and depletion of downregulated genes associated with high RET/GDNF tumors. (NS: Not significant, * $p < 0.001$).

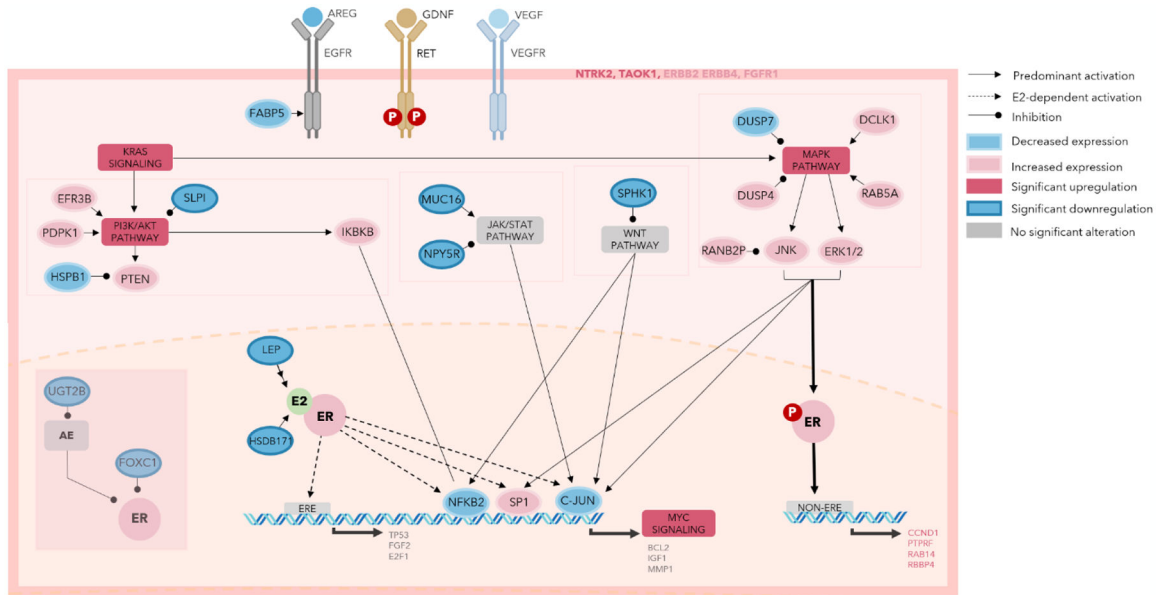


Fig. 7. Summary schematic of key receptors, pathways, and interactions in high RET/GDNF tumors.

Table 1.

Demographic and clinical features across ER+ patients with high RET/GDNF relative to all other ER+ patients.

Characteristic	Overall ER+ N = 794 ^I	High RET/GDNF N = 56 ^I	Non-high RET/GDNF N = 738 ^I	p-value ²
Age	59.29 (13.28)	60.32 (14.17)	59.21 (13.22)	0.574
Age Group				0.8
<30	7 (0.9%)	1 (1.8%)	6 (0.8%)	
30–39	43 (5.4%)	3 (5.4%)	40 (5.4%)	
40–49	148 (19%)	8 (14%)	140 (19%)	
50–59	201 (25%)	13 (23%)	188 (25%)	
60–69	215 (27%)	16 (29%)	199 (27%)	
Missing	180 (23%)	15 (27%)	165 (22%)	
Sex				>0.9
Female	782 (98%)	56 (100%)	726 (98%)	
Male	12 (1.5%)	0 (0%)	12 (1.6%)	
Race				0.019 [*]
Asian	37 (4.7%)	2 (3.6%)	35 (4.7%)	
Black or African American	109 (14%)	1 (1.8%)	108 (15%)	
White	569 (72%)	48 (86%)	521 (71%)	
Missing	79 (9.9%)	5 (8.9%)	74 (10%)	
Ethnicity				0.5
Hispanic or Latino	29 (3.7%)	2 (3.6%)	27 (3.7%)	
Not Hispanic Or Latino	621 (78%)	47 (84%)	574 (78%)	
Missing	144 (18%)	7 (12%)	137 (19%)	
Histology				<0.001 [*]
Infiltrating ductal carcinoma	526 (66%)	42 (75%)	484 (66%)	
Infiltrating ductal carcinoma mixed with other types	39 (4.9%)	6 (11%)	33 (4.5%)	
Lobular carcinoma	184 (23%)	3 (5.4%)	181 (25%)	
Mucinous adenocarcinoma	15 (1.9%)	4 (7.1%)	11 (1.5%)	
Other	18 (2.3%)	1 (1.8%)	17 (2.3%)	
Missing	12 (1.5%)	0 (0%)	12 (1.6%)	
T Stage				0.093
T1	209 (26%)	10 (18%)	199 (27%)	
T2	447 (56%)	32 (57%)	415 (56%)	
T3	111 (14%)	9 (16%)	102 (14%)	
T4	25 (3.1%)	5 (8.9%)	20 (2.7%)	
Missing	2 (0.3%)	0 (0%)	2 (0.3%)	
N Stage				0.2

Characteristic	Overall ER+ N = 794 ¹	High RET/GDNF N = 56 ¹	Non-high RET/GDNF N = 738 ¹	p-value ²
N0	351 (44%)	18 (32%)	333 (45%)	
N1	277 (35%)	28 (50%)	249 (34%)	
N2	89 (11%)	6 (11%)	83 (11%)	
N3	59 (7.4%)	3 (5.4%)	56 (7.6%)	
Missing	18 (2.3%)	1 (1.8%)	17 (2.3%)	
M Stage				0.3
M0	643 (81%)	50 (89%)	593 (80%)	
M1	15 (1.9%)	0 (0%)	15 (2.0%)	
Missing	136 (17%)	6 (11%)	130 (18%)	
AJCC Pathological Stage				0.6
Stage I	139 (18%)	7 (12%)	132 (18%)	
Stage II	431 (54%)	30 (54%)	401 (54%)	
Stage III	191 (24%)	18 (32%)	173 (23%)	
Stage IV	14 (1.8%)	0 (0%)	14 (1.9%)	
Missing	19 (2.4%)	1 (1.8%)	18 (2.4%)	
PR Status				0.2
Positive	668 (84%)	48 (86%)	620 (84%)	
Negative	122 (15%)	7 (12%)	115 (16%)	
Indeterminate	3 (0.4%)	1 (1.8%)	2 (0.3%)	
Missing	1	0	1	
HER2 Status				0.4
Positive	120 (15%)	9 (16%)	111 (15%)	
Negative	659 (83%)	45 (80%)	614 (83%)	
Indeterminate	14 (1.8%)	2 (3.6%)	12 (1.6%)	
Missing	1	0	1	
PAM50 Subtype				0.3
Luminal A	525 (66%)	34 (61%)	491 (67%)	
Luminal B	196 (25%)	20 (36%)	176 (24%)	
HER2-Enriched	29 (3.7%)	2 (3.6%)	27 (3.7%)	
Basal	20 (2.5%)	0 (0%)	20 (2.7%)	
Claudin-Low	1 (0.1%)	0 (0%)	1 (0.1%)	
Normal-Like	23 (2.9%)	0 (0%)	23 (3.1%)	

¹n (%), mean (sd)

²Fisher's exact test; Pearson's Chi-squared test

* Statistically significant