The relaxin family peptide receptor 1, RXFP1, is differentially expressed in non-small cell lung cancer and associates with patient survival.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States¹. We mined published microarray data^{2,3,4} to identify differentially expressed genes in NSCLC. We found that the gene encoding the relaxin family peptide receptor 1, RXFP1, was among those whose expression was most different in human NSCLC tumors as compared to the lung. RXFP1 expression levels were significantly decreased in NSCLC tumors relative to normal lung tissue, and lower expression of RXFP1 in patient tumors was significantly associated with decreased overall survival. RXFP1 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: RXFP1, relaxin family peptide receptor 1, LGR7, NSCLC, non-small cell lung cancer, systems biology of NSCLC, targeted therapeutics in NSCLC.

1 In 2016, lung cancer resulted in the death of 158,000 Americans; 81% of all patients
2 diagnosed with lung cancer will expire within 5 years⁵. Non-small cell lung cancer (NSCLC) is
3 the most common type of lung cancer, diagnosed in 84% of patients with lung cancer, and 76%
4 of all patients with NSCLC will expire within 5 years⁵. The rational development of targeted
5 therapeutics to treat patients with NSCLC can be supported by an enhanced understanding of
6 fundamental transcriptional features of NSCLC tumors. To discover genes associated with
7 NSCLC tumors in an unbiased fashion and at the systems-level, we mined independently
8 published microarray data^{2,3,4} to compare global gene expression profiles of NSCLC tumors to
9 that of the normal lung. We found recurrent and significant differential expression of RXFP1 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting RXFP1 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets, GSE43458², GSE33532³ and GSE74706⁴ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE43458
15 was generated using Affymetrix Human Gene 1.0 ST Array technology; for this analysis, we
16 used with $n=30$ control lung tissue and $n=80$ NSCLC tumors, and the analysis was performed
17 using platform GPL6244. GSE33532 was generated using Affymetrix Human Genome U133
18 Plus 2.0 Array technology; for this analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC
19 tumors, and the analysis was performed using platform GPL570. GSE74706 was generated
20 using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for this

analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was performed using platform GPL13497. All tumors utilized for differential gene expression analysis here were of the adenocarcinoma type.

The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether RXFP1 expression was significantly between normal lung tissue and NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of RXFP1 mRNA expression levels with overall survival in $n=1144$ non-small cell lung cancer patients.

Results

We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to discover in an unbiased fashion and at the transcriptome-level the most striking gene expression features of NSCLC tumors.

RXFP1 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding the relaxin family peptide receptor 1, RXFP1, in NSCLC tumors when compared to the lung² (Table 1). When

1 sorting each of the transcripts measured by microarray based on significance of difference in
2 RXFP1 expression between NSCLC tumors and the normal lung, in this dataset, RXFP1 ranked
3 137 out of 25906 total transcripts, equating to 99.5% differential expression (Table 1).
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5 Differential expression of RXFP1 in NSCLC tumors was statistically significant (Table 1;
6 $p=7.97E-23$).
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8 We queried a second microarray dataset³ to determine if we could validate differential
9 expression of RXFP1 in non-small cell lung cancers (Table 2). We found significant differential
10 expression of RXFP1 in NSCLC tumors of the adenocarcinoma type when compared to the
11 normal lung (Table 2). When sorting each of the transcripts measured based on significance of
12 difference in expression of RXFP1 between NSCLC tumors and the normal lung, RXFP1 ranked
13 97 of 33252 total transcripts (Table 2), equating to 99.7% differential expression. Differential
14 expression of RXFP1 in NSCLC tumors was statistically significant (Table 2; $p=5.44E-15$).
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17 Analysis of a third microarray dataset⁴ again revealed significant differential expression
18 of RXFP1 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
19 measured based on significance of difference in expression of RXFP1 between NSCLC tumors
20 and the normal lung, RXFP1 ranked 117 out of 34183 total transcripts (Table 3), equating to
21 99.7% differential expression. Differential expression of RXFP1 in NSCLC tumors was
22 statistically significant (Table 3; $p=2.6E-12$).
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26 RXFP1 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.
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28 We obtained exact mRNA levels for RXFP1 from NSCLC tumors and from the lung to

1 directly compare RXFP1 expression between tumor and control lung tissue and assess for
2 statistical significance. RXFP1 was expressed at significantly lower levels in NSCLC tumors as
3 compared to the normal lung in each dataset queried, respectively (Figure 1: $p<0.0001$, Figure 2:
4 $p<0.0001$ and Figure 3: $p<0.0001$). We calculated a mean fold change of 0.7415 ± 0.0940 and
5 0.5235 ± 0.0822 in RXFP1 expression when comparing NSCLC tumors to the lung (Table 1 and
6 Table 2, respectively).

7 RXFP1 expression in NSCLC tumors correlates with overall survival.

8 We performed Kaplan-Meier survival analysis using RXFP1 mRNA expression in
9 NSCLC tumors coupled with paired overall survival data from each patient, in 1144 NSCLC
10 patients in total, to determine whether RXFP1 tumor expression was correlated with survival
11 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of RXFP1
12 possessed significantly shorter overall survival than patients with high tumor expression of
13 RXFP1 (Figure 3). Median overall survival (OS) of patients in the low expression cohort was 48
14 months, while median OS in patients in the high RXFP1 expression cohort was 98.5 months
15 (Table 4); this difference in median OS based on RXFP1 tumor expression in NSCLC was
16 statistically significant (Figure 3; logrank p -value: $4.8e-09$, hazard ratio: 0.61 (0.52 -0.72); false
17 discovery rate=0.01).

18 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
19 differential expression of RXFP1 as among the most significant transcriptional features of
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1 NSCLC tumors, and RXFP1 expression was significantly correlated with patient outcomes, as
2 patients with lower tumor expression of RXFP1 possessed significantly worse overall survival.
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4 **Discussion**

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6 The relaxin family peptide receptor 1, RXFP1 belongs to the C subfamily of the leucine-
7 rich repeat-containing G protein-coupled receptors (LGRs) and is designated LGR7^{7,8}. The
8 ligand for RXFP1 is relaxin^{7,8}. LGR-C family members contain a low density lipoprotein
9 module at the amino terminus which has functions in maturation in the receptor and signal
10 transduction induced by ligand-binding^{7,8}. Upon activation by relaxin binding, RXFP1 is
11 internalized through beta-arrestin-dependant mechanisms and subsequently transported to the
12 membrane^{7,8}. Cells with endogenous expression of RXFP1 display complex pathway kinetics
13 involving phosphoinositide-3-kinase and protein kinase C ζ and generation of cyclic AMP
14 (cAMP) . In HEK293T cells, this signal is transduced initially through $G\alpha_s$ and later through G-
15 $\beta\gamma$ of $G\alpha_{i3}$, with an inhibitor pathway mediated by $G\alpha_{oB}$ ⁹. RXFP1 homodimerizes in the
16 endoplasmic reticulum and dimerization is necessary for trafficking to the cell surface; one study
17 reported that the LDL-A module of RXFP1 did not form dimers¹⁰.
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22 Relaxin has physiologic functions including increased cardiac output, decreased vascular
23 resistance, increased arterial compliance and renal blood flow during pregnancy¹¹. The
24 concentration of relaxin increases in the blood during pregnancy in the first trimester and it can
25 induce softening of the cervix during parturition¹². A small-molecule agonist of the relaxin
26 receptor was identified and developed using high-throughput screening, with the ability to induce
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1 increased cAMP concentrations in HEK293T¹³. Relaxin can induce gene expression of the
2 vascular endothelial growth factor, and small molecule agonists of RXFP1 could similarly induce
3 VEGF expression in THP1 cells¹³. It has been suggested that relaxin can function as an activator
4 of the peroxisome proliferator-activated receptor gamma (PPAR γ) in a mechanism involving
5 RXFP1 but independent of PPAR γ ligand-binding¹⁴.

8 In the context of the lung, expression of RXFP1 is decreased in the lung tissues of patients
9 with idiopathic pulmonary fibrosis, and magnitude of RXFP1 reduction correlated with carbon
10 monoxide diffusing capacity in the lung¹⁵. Transforming growth factor- β (TGF- β) could
11 decrease RXFP1 expression in lung fibroblasts from diseased and control subjects¹⁵. Down-
12 regulation of RXFP1 in IPF was attributed to expression of microRNA miR-144-3p, which was
13 TGF- β -inducible¹⁶. In cancer, one study reported expression of RXFP1 in glioblastoma tumor
14 tissue but not in normal astrocytes, suggesting that RXFP1 could function in resistance to
15 temozolomide by inducing base excision repair and inhibition caspase 3/7-mediated apoptosis¹⁷.

19 We found, by mining multiple independently published microarray datasets, that the
20 relaxin family peptide receptor 1, RXFP1, was among the genes most differentially expressed in
21 the primary tumors of patients with non-small cell lung cancer; RXFP1 was expressed at
22 significantly lower levels in NSCLC tumors as compared to the lung, and decreased expression
23 of RXFP1 was significantly associated with worse overall survival in NSCLC patients. RXFP1
24 may be of relevance to the biology of tumor initiation or progression in patients with NSCLC of
25 the adenocarcinoma type, the most common type of the leading cause of cancer death in the
26 United States and worldwide.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
137	8098060	7.97E-23	-12.454607	41.380871	0.7415 ± 0.0940	RXFP1	relaxin/insulin like family peptide receptor 1

Table 1: RXFP1 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, fold change of RXFP1 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
97	238206_at	5.44E-15	-14.030035	24.2115711	0.5235 ± 0.0822	RXFP1	relaxin/insulin like family peptide receptor 1

Table 2: RXFP1 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change of RXFP1 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
117	A_23_P433050	2.6E-12	-1.15E+01	18.1602347	RXFP1

Table 3: RXFP1 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared and gene are listed in this chart.

Figure 1: RXFP1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of RXFP1 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in RXFP1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: RXFP1 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of RXFP1 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in RXFP1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: RXFP1 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of RXFP1 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in RXFP1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: RXFP1 expression in NSCLC tumors significantly correlates with overall survival.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1144$ total patients stratified into two groups, based on low or high expression of RXFP1 in patient tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on RXFP1 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
48	98.5

Table 4: Median overall survival of NSCLC patients is greater in patients with high tumor expression of RXFP1.

The median overall survival of $n=1144$ NSCLC patients based on stratification into low or high expression of RXFP1 in tumors is listed in this chart.