The faciogenital dysplasia gene FGD5 is differentially expressed in non-small cell lung cancer and associates with patient survival.

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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 faciogenital dysplasia gene 5, FGD5, was among the genes whose expression was most quantitatively different in tumors from patients with NSCLC as compared to the lung. FGD5 expression was significantly decreased in NSCLC tumors as compared to the lung, and lower expression of FGD5 in patient tumors was significantly associated with worse overall survival. FGD5 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: FGD5, 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 FGD5 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting FGD5 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets GSE33532³, GSE43458⁴ and GSE74706⁵ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE33532
15 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology; for this
16 analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was
17 performed using platform GPL570. GSE43458 was generated using Affymetrix Human Gene
18 1.0 ST Array technology; for this analysis, we used with $n=30$ control lung tissue and $n=80$
19 NSCLC tumors, and the analysis was performed using platform GPL6244. GSE74706 was
20 generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for

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

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5 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
6 differential expression but raw p -values were used to assess statistical significance of global
7 differential expression. Log-transformation of data was auto-detected, and the NCBI
8 generated category of platform annotation was used. A statistical test was performed to evaluate
9 whether FGD5 expression was significantly between normal lung tissue and NSCLC tumors
10 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
11 analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-
12 Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of FGD5
13 mRNA expression levels with overall survival in $n=1144$ non-small cell lung cancer patients.
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19 **Results**

20 We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to
21 discover in an unbiased fashion and at the transcriptome-level the most striking gene expression
22 features of NSCLC tumors.
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25 FGD5 is differentially expressed in non-small cell lung cancers.

26 We found significant differential expression of the faciogenital dysplasia gene FGD5 in
27 NSCLC tumors when compared to the lung² (Table 1). When sorting each of the transcripts
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measured based on significance of difference in expression of FGD5 between NSCLC tumors and the normal lung, FGD5 ranked 21 out of 25906 total transcripts (Table 1). Differential expression of FGD5 in NSCLC tumors was statistically significant (Table 1; $p=2.82E-17$).

We queried a second microarray dataset⁴ to determine if we could validate differential expression of FGD5 in non-small cell lung cancers (Table 2). We again found significant differential expression of FGD5 in NSCLC tumors of the adenocarcinoma type when compared to the normal lung (Table 2). When sorting each of the transcripts measured based on significance of difference in expression of FGD5 between NSCLC tumors and the normal lung, FGD5 ranked 153 out of 33252 total transcripts (Table 2). Differential expression of FGD5 in NSCLC tumors was statistically significant (Table 2; $p=1.98E-22$).

Analysis of a third microarray dataset³ revealed significant differential expression of FGD5 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts measured based on significance of difference in expression of FGD5 between NSCLC tumors and the normal lung, in this dataset, FGD5 ranked 45 out of 34183 total transcripts (Table 3). Differential expression of FGD5 in NSCLC tumors was statistically significant (Table 3; $p=1.76E-13$).

FGD5 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.

We obtained exact mRNA levels for FGD5 from NSCLC tumors and from the lung to directly compare FGD5 expression between tumor and control lung tissue and assess for statistical significance. FGD5 was expressed at significantly lower levels in NSCLC tumors as

1 compared to the normal lung in each dataset queried (Figure 1: $p<0.0001$, Figure 2: $p<0.0001$,
2 Figure 3: $p<0.0001$). We calculated a mean fold change of 0.7706 ± 0.0403 (Table 1) and
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4 0.8383 ± 0.0702 (Table 2) in FGD5 expression when comparing NSCLC tumors to the lung.

6 FGD5 expression in NSCLC tumors correlates with overall survival.

7 We performed Kaplan-Meier survival analysis using FGD5 mRNA expression in NSCLC
8 tumors coupled with paired overall survival data from each patient, in 1144 NSCLC patients in
9 total, to determine whether FGD5 tumor expression was correlated with survival outcomes in
10 NSCLC. We found that patients whose tumors expressed lower levels of FGD5 possessed
11 significantly shorter overall survival than patients with high tumor expression of FGD5 (Figure
12 4). Median overall survival (OS) of patients in the low expression cohort was 18.77 months,
13 while median OS in patients in the high FGD5 expression cohort was 54.17 months (Table 4);
14 this difference in median OS based on FGD5 tumor expression in NSCLC was statistically
15 significant (Figure 4; logrank p -value: $6.4e-14$; hazard ratio: 0.44 (0.35 -0.55)).
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20 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
21 differential expression of FGD5 as among the most significant transcriptional features of NSCLC
22 tumors, and FGD5 expression was significantly correlated with patient outcomes, as patients
23 with lower tumor expression of FGD5 possessed significantly worse overall survival.
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Discussion

The faciogenital dysplasia gene 5, FGD5, was identified based on expression in precursor cells of the endothelium in mouse embryos and in the vasculature of adult mice as well as zebrafish larvae. The FGD family of molecules are Rho guanine-nucleotide exchange factors (GEFs) that all contain pleckstrin homology (PH) and FYVE domains. Multiple lines of evidence support a role for FGD5 in control of vascularization. In the mouse embryo, FGD5 displays specific expression in Flk1+ cells, with expression observable as early as embryonic day 8.5 (E8.5)⁷. Enrichment of endothelial precursors based on Flk1 expression demonstrated increased expression of genes involved in angiogenesis including Tie1 and angiopoietin 2. FGD5 can function in pruning of neovessels, a process known as vascular regression, during the generation of new blood vessels, or neovascularization⁷. In human umbilical vein endothelial cells (HUVEC), depletion of FGD5 *in vitro* resulted in increased cord extension, while ectopic expression of FGD5 impaired cord formation⁷. *In vivo*, a role for FGD5 in inhibition of angiogenesis was supported by a cell-bead plug assay in severe combined immunodeficiency (SCID) mice, with a decrease in HUVEC accumulation on beads resulting from adenoviral-expression of FGD5⁷. At the protein level, FGD5 can interact with cdc42 and promotion of cell death of endothelial cells by apoptosis through Hey1, p53, and p21^{CIP1}⁷. In HUVEC, FGD5 can localize at ruffles of the plasma membrane and in perinuclear regions. Depletion of FGD5 results in impairment of Cdc42 activation by VEGF, as well inhibition of VEGF-induced permeability, proliferation and Golgi apparatus orientation⁸. FGD5 can interact with VEGFR2, promoting its localization to endosomes, association with phosphoinositide-3 kinase (PI3 kinase)

1 while preventing its lysosomal degradation⁹. In human endothelial cell spheroids depleted of
2 FGD5, filopodia formation is impaired as well as angiogenic sprouting induced by VEGF in
3 conjunction with loss of expression of DLL4, VEGFR2 and CXCR4, key markers of tip cell
4 gene expression program⁰. The protein tyrosine phosphatase VE-PTP can dephosphorylate
5 FGD5, and accordingly, inhibition of VE-PTP in conjunction with Tie-2 activation results in
6 phosphorylation of FGD5 at tyrosine 820¹⁰. The phenotypic consequence of FGD5-Y820 is
7 stabilization of endothelial cell junctions, important for vascular permeability¹⁰. Analysis of
8 FGD5-deficient mice revealed that although FGD5 is not required for hematopoietic stem cell
9 function or for definitive hematopoiesis, isolation of bone marrow cells based on FGD5 could
10 produce a fraction with equivalent HSC activity to that of LSKCD150⁺CD48⁻ (HSC^{slam})¹⁰. Thus,
11 FGD5 expression marks immunophenotypic and functionally competent murine hematopoietic
12 stem cells¹¹.

17 FGD5 is implicated in a number of human cancers. A study of 430 breast tumors found
18 that FGD1 was amplified in 9.5% of all breast cancers surveyed, with higher cumulative risk of
19 death from breast cancer among cases with FGD5 amplification¹². In a separate study of 829
20 primary breast cancers, proportion of cancers with FGD5 expression was significantly higher
21 with in patients with metastasis to the lymph nodes¹³. There was also some association between
22 FGD5 amplification and nuclear localization of FGD5¹³. An anti-sense non-coding RNA,
23 FGD5-AS1 is described as possessing perturbed expression in multiple cancers, including in
24 colorectal cancer¹⁴ and in non-small cell lung cancer (NSCLC)^{15,16}. In colorectal cancer,
25 expression of FGD5-AS1 was significantly increased in colorectal cancer cell lines and its
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1 depletion resulted in impaired proliferation and migration *in vitro*¹⁴. Mechanistic studies
2 demonstrated that FGD5-AS1 could interact with and serve as a sponge for the microRNA
3 miR-302e¹⁴. In tumor tissues from patients with NSCLC and in NSCLC cell lines NCI-H1703,
4 NCI-H1793 and NCI-H1869, expression of FGD5-AS1 was significantly increased as compared
5 to non-tumor tissue or the IMR-90 cell line lung fibroblast cell line, respectively¹⁵. Depletion of
6 FGD5 significantly impaired proliferation and colony formation of NSCLC cell lines *in vitro* and
7 increased cell death¹⁵. Here, FGD5-AS1 functioned to sponge another microRNA, miR-107,
8 serving to liberate a miR-107 target, FGFR1 and thereby enhance cell proliferation¹⁵. Finally, a
9 study of cisplatin resistance in NSCLC found that expression of both FGD-AS1 and WEE1 was
10 up-regulated in cisplatin-resistant tumors; depletion of FGD5-AS1 impaired cell proliferation
11 and migration in cisplatin-resistant NSCLC cells *in vitro* and reduced tumor growth in cisplatin-
12 resistant mice¹⁶. Here, FGD5-AS1 exerted its influence through interaction with microRNA
13 miR-140-5p to enhance WEE1 expression¹⁶. Thus, expression of FGD5 or non-coding RNAs
14 transcribed from the FGD5 locus is perturbed in multiple human cancers.

20 We found, by mining multiple independently published microarray datasets, that FGD5
21 was among the genes most differentially expressed in the primary tumors of patients with non-
22 small cell lung cancer; FGD5 was expressed at significantly lower levels in NSCLC tumors
23 as compared to the lung, and decreased expression of FGD5 was significantly associated with
24 worse overall survival in NSCLC patients. FGD5 may be of relevance to the biology of tumor
25 initiation or progression in patients with NSCLC of the adenocarcinoma type, the most common
26 type of the leading cause of cancer death in the United States and worldwide.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
21	226985_at	2.82E-17	-16.978625	29.367712	0.7706 ± 0.0403	FGD5	FYVE, RhoGEF and PH domain containing 5

Table 1: FGD5 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 FGD5 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
153	8078066	1.98E-22	-12.281167	40.484043	0.8383 ± 0.0702	FGD5	FYVE, RhoGEF and PH domain containing 5

Table 2: FGD5 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 FGD5 expression in NSCLC tumors as compared to the lung, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
45	A_33_P3364180	1.76E-13	-1.28E+01	20.788309	FGD5

Table 3: FGD5 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, gene and gene name are listed in this chart.

Figure 1: FGD5 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FGD5 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 FGD5 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: FGD5 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FGD5 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 FGD5 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: FGD5 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FGD5 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 FGD5 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: FGD5 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 FGD5 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 FGD5 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)
18.77	54.17

Table 4: FGD5 expression in NSCLC tumors significantly correlates with patient survival.

The median overall survival of $n=1144$ NSCLC patients based on stratification into low or high expression of FGD5 in tumors is listed in this chart.