Pyrroline-5-carboxylate reductase 1, PYCR1, 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 pyrroline-5-carboxylate reductase 1, PYCR1, was among those whose expression was most quantitatively different in human NSCLC tumors as compared to the lung. PYCR1 expression levels were significantly increased in NSCLC tumors as compared to the lung, and higher expression of PYCR1 in patient tumors was significantly correlated with worse overall survival and decreased time to first progression. PYCR1 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: PYCR1, pyrroline-5-carboxylate reductase 1, 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 PYCR1 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting PYCR1 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets, GSE43458², GSE33532⁴ 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 PYCR1 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 PYCR1 mRNA expression levels with overall survival in $n=1925$ non-small cell lung cancer patients and with time to first progression in $n=982$ NSCLC 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.

PYCR1 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding pyrroline-5-carboxylate

1 reductase 1, PYCR1, in NSCLC tumors when compared to the lung² (Table 1). When sorting
2 each of the transcripts measured based on significance of difference in expression of PYCR1
3 between NSCLC tumors and the normal lung, in this dataset, PYCR1 ranked 279 out of 25906
4 total transcripts (Table 1), equating to 98.9% differential expression. Differential expression of
5 PYCR1 in NSCLC tumors was statistically significant (Table 1; $p=2.28E-19$).
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8 We queried a second microarray dataset³ to determine if we could validate differential
9 expression of PYCR1 in non-small cell lung cancers (Table 2). We found significant differential
10 expression of PYCR1 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 PYCR1 between NSCLC tumors and the normal lung, PYCR1 ranked
13 255 of 33252 total transcripts (Table 2), equating to 99.2% differential expression. Differential
14 expression of PYCR1 in NSCLC tumors was statistically significant (Table 2; $p=5.7E-13$).
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16
17 Analysis of a third microarray dataset⁴ again revealed significant differential expression
18 of PYCR1 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
19 measured based on significance of difference in expression of PYCR1 between NSCLC tumors
20 and the normal lung, PYCR1 ranked 217 out of 34183 total transcripts (Table 3), equating to
21 99.4% differential expression. Differential expression of PYCR1 in NSCLC tumors was
22 statistically significant (Table 3; $p=2.96E-11$).
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25
26 PYCR1 is expressed at significantly higher levels in NSCLC tumors as compared to the lung.
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28 We obtained exact mRNA levels for PYCR1 from NSCLC tumors and from the lung to

1 directly compare PYCR1 expression between tumor and control lung tissue and assess for
2 statistical significance. PYCR1 was expressed at higher levels in NSCLC tumors as
3 compared to the normal lung, and this difference in PYCR1 mRNA expression between NSCLC
4 tumors and normal lung tissue was statistically significant (Figure 1: $p < 0.0001$).

7 PYCR1 expression in NSCLC tumors correlates with overall survival and time to first
8 progression.

9 We performed Kaplan-Meier survival analysis using PYCR1 mRNA expression in
10 NSCLC tumors coupled with paired overall survival data from each patient, in 1925 NSCLC
11 patients in total, to determine whether PYCR1 tumor expression was correlated with survival
12 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of PYCR1
13 possessed significantly shorter overall survival than patients with high tumor expression of
14 PYCR1 (Figure 2). Median overall survival (OS) of patients in the low expression cohort was 76
15 months, while median OS in patients in the high PYCR1 expression cohort was 52 months
16 (Table 4); this difference in median OS based on PYCR1 tumor expression in NSCLC was
17 statistically significant (Figure 2; logrank p -value: 2.4×10^{-5} ; hazard ratio: 1.32 (1.16 - 1.51)).

21 Kaplan Meier also demonstrated that PYCR1 tumor mRNA expression was significantly
22 correlated with time to first progression (FP) in 982 patients with non-small cell lung cancer.
23 Median FP for patients with low tumor expression of PYCR1 was 45.3 months while median FP
24 for patients with high tumor expression of PYCR1 was 13.63 months (Table 5). The difference in
25 median FP based on PYCR1 tumor expression in NSCLC was statistically significant (Figure 3;
26 logrank p -value: 1.3×10^{-10} ; hazard ratio: 2.25 (1.74 - 2.9)).

Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed differential expression of PYCR1 as among the most significant transcriptional features of NSCLC tumors, and PYCR1 expression was significantly correlated with patient outcomes, as patients with higher tumor expression of PYCR1 possessed significantly worse overall survival and shorter time to first progression.

Discussion

Pyrroline-5-carboxylate reductase functions in the proline biosynthetic pathway by catalyzing conversion of P5C intermediate to proline. PYCR1 is utilized by a DNA tumor virus during transformation, demonstrating its importance in cancer. The K1 oncoprotein of the Kaposi's sarcoma herpesvirus functions, at least in part, through interactions with PYCR1⁷. K1 interactions with PYCR1 result in PYCR1 activation and biosynthesis of proline, and in a 3-dimensional cell culture system but not in standard cell culture (2-dimensional), PYCR1 was required for tumor growth induced by K1⁷. Moreover, expression of the oncoprotein Myc was sufficient to support biosynthesis of proline, and Myc could function in transcriptional activation of PYCR1⁸. Outside of cancer, patients with cutis laxa, a condition of the skin featuring loose, redundant tissue and hypo-elastic skin is caused by mutations in PYCR1⁹.

Perturbed expression of PYCR1 has been implicated in a number of human cancers. A human anaplastic oligodendroglioma cell line containing a mutation in isocitrate dehydrogenase (R132H) could support synthesis of proline from glutamine, and this was found to be mediated by PYCR1¹⁰. One study described copy numbers gains in the PYCR1 gene in 24.7% of luminal B breast cancers¹¹. PYCR1 expression was found to be up-regulated in invasive breast

1 carcinomas, and deletion of PYCR1 in vivo in breast cancer xenograft mouse models resulted in
2 reduction of tumor growth, further evidence of the importance of PYCR1 in at least some breast
3 cancers¹². In the 4T1 and EMT6.5 orthotopic mouse models of breast cancer, inhibition of the
4 enzyme that antagonizes PYCR1 function, proline dehydrogenase (PRODH) resulted in
5 reduction of metastasis to the lungs¹³. Up-regulation of PYCR1 expression has also been
6 described in clear cell renal carcinomas¹². Two studies describe roles for PYCR1 in non-small
7 cell lung cancer. PYCR1 was found to be expressed at significantly higher levels in most, but
8 not all NSCLC tumors as compared to adjacent normal tissue, PYCR1 expression could support
9 proliferation of the non-small cell lung cancer cell lines H1703 and SPC-A1¹⁴, and survival after
10 surgery was significantly longer in patients with low tumor expression of PYCR1. Depletion of
11 PYCR1 resulted in induction of apoptosis in SPC-A1 cells but not H1703 cells, and PYCR1
12 depletion resulted in decreased expression of Cyclin D1, Bcl-2 and Bcl-XL. A separate study
13 found that PYCR1 over-expression in A549 NSCLC cells promoted colony formation *in vitro*
14 and that the microRNA miR-488 could negatively regulate PYCR1 expression in A549 cells¹⁵.

20 We found, by mining multiple independently published microarray datasets, that
21 PYCR1 was among the genes most differentially expressed in the primary tumors of patients
22 with non-small cell lung cancer; PYCR1 was expressed at significantly higher levels in NSCLC
23 tumors as compared to the lung, and patients with high PYCR1 tumor expression had worse
24 overall survival and shorter time to first progression. PYCR1 may be of relevance to the biology
25 of tumor initiation or progression in patients with NSCLC of the adenocarcinoma type, the most
26 common type of the leading cause of cancer death in the United States and worldwide.

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Rank	ID	p-value	t	B	Gene	Gene name
279	8019316	2.28E-19	10.946679	33.524144	PYCR1	pyrroline-5-carboxylate reductase 1

Table 1: PYCR1 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 PYCR1 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	Gene name
255	202148_s_at	5.7E-13	11.754329	19.6023347	PYCR1	pyrroline-5-carboxylate reductase 1

Table 2: PYCR1 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.

Rank	ID	p-value	t	B	Gene
217	A_23_P130194	2.96E-11	1.04E+01	15.7693534	PYCR1

Table 3: PYCR1 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 PYCR1 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Figure 1: PYCR1 is expressed at significantly higher levels in NSCLC tumors when compared to the lung.

The mRNA expression level of PYCR1 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 PYCR1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: PYCR1 expression in NSCLC tumors significantly correlates with overall survival.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1925$ total patients stratified into two groups, based on low or high expression of PYCR1 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 PYCR1 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)
76	52

Table 4: PYCR1 expression in NSCLC tumors significantly correlates with patient survival.

The median overall survival of $n=1925$ NSCLC patients based on stratification into low or high expression of PYCR1 in tumors is listed in this chart.

Figure 3: PYCR1 expression in NSCLC tumors significantly correlates with time to first progression.

Depicted in this Kaplan-Meier plot is time to first progression for $n=982$ total patients stratified into two groups, based on low or high expression of PYCR1 in patient tumors. The log rank p -value denoting statistical significance of difference in time to first progression 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 PYCR1 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)
45.3	13.63

Table 5: PYCR1 expression in NSCLC tumors significantly correlates with time to first progression.

The median time to first progression of $n=982$ NSCLC patients based on stratification into low or high expression of PYCR1 in tumors is listed in this chart.