Comprehensive characterization of interleukin-enhanced factor 2 (ILF2) in triple-negative breast cancer (TNBC)

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BACKGROUND

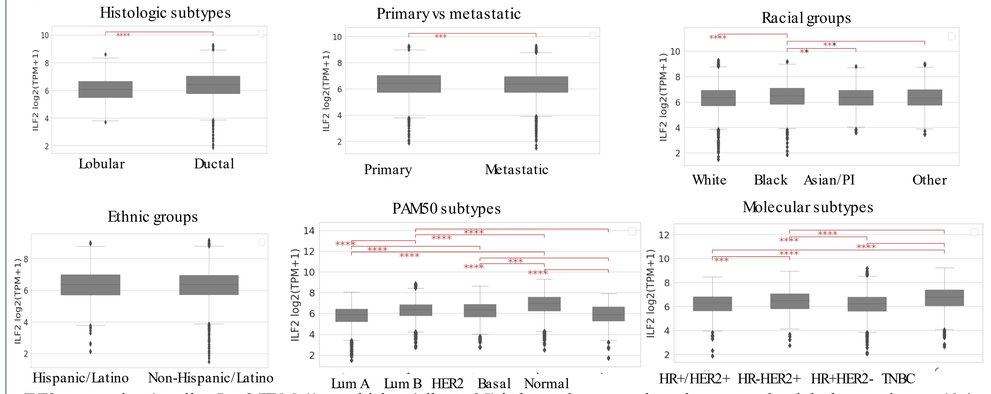
- While treatment and management of TNBC has improved, there is a need for novel prognostic biomarkers to better inform outcomes and guide therapeutic options.
- ILF2 is a poorly characterized protein with pleiotropic functions that is highly expressed in TNBC.
- Here we evaluated the associations of ILF2 with 1) genomic and transcriptomic data, 2) tumor microenvironment (TME), and 3) clinical outcomes in TNBC.

METHODS

- 15,544 breast cancer (BC) samples, including 3,038 TNBC, were tested by NGS (592,NextSeq; WES, NovaSeq) and WTS (NovaSeq; Caris Life Sciences, Phoenix, AZ).
- TNBC ILF2-high(H) and ILF2-low(L) RNA expression were classified as top 25% and bottom 25% quartile, respectively.
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Real-world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates.
- Statistical significance was assessed using chi-square and Mann-Whitney U tests with multiple comparison adjustments (q < 0.05).

RESULTS

Figure 1. Analysis of ILF2 expression



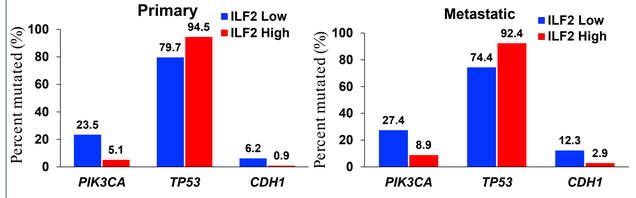
ILF2 expression (median Log2(TPM+1) was higher (all q < .05) in key subgroups: ductal compared to lobular carcinoma (6.4 vs 6.0); primary compared to metastatic BC (6.4 vs 6.3);African American compared to White (6.4 vs 6.3); basal compared to luminal A, luminal B,HER2 PAM50 subtypes (6.9 vs 5.8, 6.3, 6.3); and TNBC compared to HR+HER2+, HR-HER2+,HR+HER2- subtypes (6.7 vs 6.3, 6.4, 6.2)

Table 1: TNBC patients' sample demographic information

	Primary		Metastatic	
Variables	ILF2 low (25th percentile)	ILF2 high (25th percentile)	ILF2 low (25th percentile)	ILF2 high (25th percentile)
Count (N)	347	391	392	348
Age				
<50	19.88%(69/347)	35.55%(139/391)	17.35%(68/392)	28.16%(98/348)
≥50	80.12%(278/347)	64.45%(252/391)	82.65%(324/392)	71.84%(250/348)
Histological subtypes (count, N)				
Lobular	4.03%(14/347)	0.77%(3/391)	2.81%(11/392)	0.29%(1/348)
Ductal	64.27%(223/347)	75.19%(294/391)	9.95%(39/392)	15.23%(53/348)
Other/Unclear	31.7%(110/347)	24.04%(94/391)	87.24%(342/392)	84.48%(294/348)
Race (count, N)				
White	59.85%(158/264)	60%(165/275)	63.35%(204/322)	57.68%(154/267)
Black	31.44%(83/264)	33.82%(93/275)	25.78%(83/322)	30.34%(81/267)
Asian/Pacific Islander	3.79%(10/264)	2.91%(8/275)	4.66%(15/322)	3.75%(10/267)
Other	4.92%(13/264)	3.27%(9/275)	6.21%(20/322)	8.24%(22/267)
Ethnicity (count, N)				
Hispanic/Latino	19.47%(51/262)	17.69%(46/260)	11.86%(35/295)	17.6%(44/250)
Not Hispanic/Latino	80.53%(211/262)	82.31%(214/260)	88.14%(260/295)	82.4%(206/250)

Race/ethnicity data is self-reported

Figure 2. Mutation analysis of ILF2-low vs high TNBC



ILF2 high had higher mutation frequency of TP53 (pTNBC: 94.5% vs 79.7%; mTNBC: 92.4% vs 74.4%), but lower frequencies for PIK3CA(pTNBC: 5.1% vs 23.5%, mTNBC: 8.9% vs 27.4%), CDH1 (pTNBC: 0.9% vs 6.2%; mTNBC: 2.9% vs 12.3%; all q <0.05)

Figure 3. Immune cell infiltration

Primary

	ILF2-L	ILF2-H	
B cell	3.83	3.89	
M1 Mφ	3.08	3.08	
M2 Mφ	3.34	2.45	*
Neutrophil	3.93	4.5	
NK cell	2.65	3.05	*
T cell CD8+	0.49	0.5	
Treg	1.96	1.54	*
DC	2.41	3.56	

Metastatic

	ILF2-L	ILF2-H	
B cell	3.91	3.86	
M1 Mφ	2.72	3.05	
M2 Mφ	3.21	2.65	*
Neutrophil	4.06	4.59	
NK cell	2.63	2.85	*
T cell CD8+	0.37	0.41	
Treg	1.79	1.44	*
DC	2.31	3.38	

Low Median% High Median%

ILF2 high had higher infiltration of NKcells (pTNBC: 3.05% vs 2.65%; mTNBC: 2.85% vs 2.63%), but lower infiltration of M2 Mφ (pTNBC: 2.45% vs 3.34%; mTNBC: 2.65% vs 3.21%) and Tregs (pTNBC: 1.54% vs 1.96%; mTNBC: 1.44% vs 1.79%; all q < .05). Proportion of M1 Mφ was similar in ILF2-H vs Lprimary TNBC.

Figure 4. Immune checkpoint gene expression

Primary

	ILF2-L	ILF2-H	
CD274	2.88	6.41	*
PDCD1	0.42	0.65	
PDCD1LG2	1.05	2.05	*
CTLA4	1.06	2.54	*
LAG3	2.33	5.39	*
HAVCR2	11.49	25.79	*
FOXP3	1.70	3.58	*

Metastatic

	ILF2-L	ILF2-H	
CD274	2.69	5.39	*
PDCD1	0.37	0.53	
PDCD1LG2	0.87	1.72	*
CTLA4	0.96	1.59	*
LAG3	2.27	4.95	*
HAVCR2	10.87	26.56	*
FOXP3	1.47	3.28	*

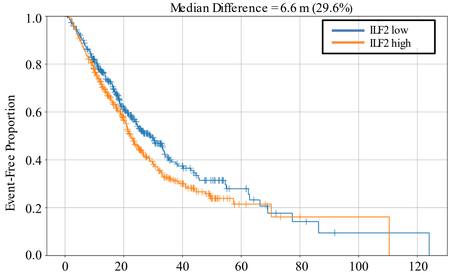
Low TPM(Median) High TPM(Median)

ILF2 high had Higher expression levels of CD274, PDCD1LG2, CTLA4, LAG3, HAVCR2, FOXP3, FC: 1.2-3.1; all q <0.05.

Figure 5 . ILF2 high vs low TNBC patient survival

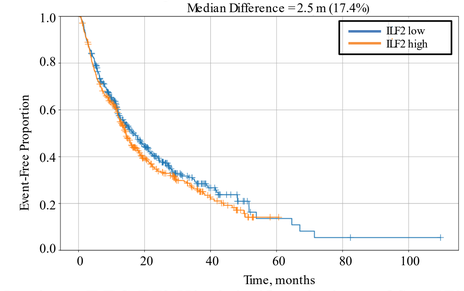
Primary

Performance: Collection -> Last Contact
HR = 0.81 (95% CI: 0.665 – 0.986) p = 0.035
ILF2 Low (341) Median = 28.9 m (95% CI: 23.8 m – 33.7 m)
ILF2 High (387) Median = 22.3 m (95% CI: 20.9 m – 25.2 m)
Median Difference = 6.6 m (29.6%)



Metastatic

Performance: Collection -> Last Contact
HR = 0.894 (95% CI: 0.748 – 1.069) p = 0.219
ILF2 Low (387) Median = 16.9 m (95% CI: 13.7 m – 19.8 m)
ILF2 High (344) Median = 14.4 m (95% CI: 12.4 m – 16.2 m)
Median Difference = 2.5 m (17.4%)



In primary TNBC, ILF2-H had significantly shorter OS vs ILF2-L group (22.3 vs 28.9 months, HR 0.81 [95% CI 0.67-0.99], p = 0.035), but no significant differences were observed between metastatic TNBC ILF2 groups (HR 0.89 [95% CI 0.75-1.07], p = 0.22).

CONCLUSIONS

ILF2-H TNBC patients showed differential genomic and transcriptomic alterations that relate to therapy resistance, immune suppressive TME, and shorter OS. Further studies are warranted to validate the effects of ILF2 upregulation on therapeutic efficacy.