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Distinct lymphocyte antigens 6 (Ly6) family members Ly6D, Ly6E, Ly6K and Ly6H drive tumorigenesis and clinical outcome

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ABSTRACT

Stem cell antigen-1 (Sca-1) is used to isolate and characterize tumor initiating cell populations from tumors of various murine models [1]. Sca-1 induced disruption of TGF-β signaling is required *in vivo* tumorigenesis in breast cancer models [2, 3-5]. The role of human Ly6 gene family is only beginning to be appreciated in recent literature [6-9]. To study the significance of Ly6 gene family members, we have visualized one hundred thirty gene expression omnibus (GEO) dataset using Oncomine (Invitrogen) and Georgetown Database of Cancer (G-DOC). This analysis showed that four different members Ly6D, Ly6E, Ly6H or Ly6K have increased gene expressed in bladder, brain and CNS, breast, colorectal, cervical, ovarian, lung, head and neck, pancreatic and prostate cancer than their normal counter part tissues. Increased expression of Ly6D, Ly6E, Ly6H or Ly6K was observed in sub-set of cancer type. The increased expression of Ly6D, Ly6E, Ly6H and Ly6K was found to be associated with poor outcome in ovarian, colorectal, gastric, breast, lung, bladder or brain and CNS as observed by KM plotter and PROGgeneV2 platform. The remarkable findings of increased expression of Ly6 family members and its positive correlation with poor outcome on patient survival in multiple cancer type indicate that Ly6 family members Ly6D, Ly6E, Ly6K and Ly6H will be an important targets in clinical practice as marker of poor prognosis and for developing novel therapeutics in multiple cancer type.

INTRODUCTION

The lymphocyte antigen-6 (Ly6) complex, a group of alloantigens, was first discovered in mice approximately 40 years ago on lymphocytes [3, 4]. Ly6 family members are evolutionary conserved and have been mapped to human chromosome 8, in particular, the 8q24.3 locus, which is syntenic to murine chromosome 15 [9, 10]. The founding Ly6 member CD59 was identified in human lymphoid cells with a role in the complement membrane attack complex and T cell activation [11]. To date, 20 human Ly6 proteins, ranging from 11-36 kDa, have been identified and categorized as either transmembrane or secretory based on the availability of a GPI-anchored signal sequence [9]. Ly6 family is located on chromosome 8q24 alongside c-Myc. The somatic copy number gain in 8q has been associated with most prevalent copy number gain in multiple cancer types [12, 13]. Ly6E and Ly6K has been implicated in development of novel therapeutics in multiple cancers [7, 8, 14, 15]. We have previously shown that increased levels of Ly6A/E (Sca-1) promote breast tumorigenesis via disruption of TGF-β signaling and suppression of GDF10 expression in mouse models [2]. GDF10 has been shown to regulate epithelial to mesenchymal transition, growth and invasion in oral squamous cell carcinoma [16]. These finding suggest that Ly6 genes family members have important role multiple cancer but a comprehensive analysis of multiple members

of Ly6 gene family and its relation to cancer patient survival is lacking.

Here we evaluate the importance and significance of novel Ly6 family in cancer prognosis and treatment using publically available datasets of gene expression micro array analysis coupled with clinical outcome information. To study the status of Ly6D, Ly6E, Ly6H and Ly6K mRNAs in human normal and cancer tissues in one-hundred and thirty gene expression omnibus (GEO) dataset using Oncomine (Invitrogen) or Georgetown Database of Cancer (G-DOC). The expression status of Ly6D, Ly6E, Ly6H and Ly6K in caner tissue was correlated with patient outcome using KM plotter and PROGgeneV2 platform.

RESULTS

Increased expression of Ly6D in multiple cancers

To examine the status of Ly6D in human cancer, we used Oncomine or G-DOC to analyze gene expression omnibus (GEO) datasets. The data summarized in Table 1 showed a significant increased expression of Ly6D in bladder cancer (n=150) than normal tissues (n=57) in Sanchez-Carbayo [17] and Dryskjot [18] studies. Ly6D mRNA expression was increased significantly in brain cancer (n=131) than normal tissues (n=23) in Sun study [19]. Ly6D mRNA expression was increased significantly in breast cancer (n=1597) than normal tissues (n=153) in Curtis study [20] and Lin study [21]. Ly6D mRNA expression was increased significantly in head and neck cancer (n=56) than normal tissues (n=41) in Estilo [22], He [23] and Frierson [24] studies. Ly6D mRNA expression was increased significantly in gastric cancer (n=31) than normal tissues (n=19) in Cho [25] study. Ly6D mRNA expression was increased significantly in lung cancer (n=453) than normal tissues (n=244) in Landi [26], Selamat [27], Su [28], Okayana [29], Bhattacharjee [30], Hou [31], Wachi [32] studies. Ly6D mRNA expression was increased significantly in ovarian cancer (n=221) than normal tissues (n=18) in Wachi [32], Welsh [33], Hendrix [34] and Bonome [35] studies. Ly6D mRNA expression was increased significantly in pancreatic cancer (n=75) than normal tissues (n=55) in Pei [36] and Badea [37] studies. Ly6D mRNA expression was increased significantly in colorectal cancer (n=369) than normal tissues (n=150) in The Cancer Genome Atlas (TCGA), Sabates-Bellver [38], Kaiser [39], Gaedcke [40] and Skrzypczak [41] studies. Ly6D mRNA was increased significantly in Kidney cancer (n=53) than normal tissues (n=28) in Jones [42] and Yusenko [43] studies.

These results show that Ly6D expression was significantly increased in bladder, brain and CNS, breast, head and neck, gastric, lung, ovarian, pancreatic, colorectal and kidney cancer than their counterpart normal tissues.

The data summarized in Table 2 showed that Ly6D mRNA expression was increased significantly in subtypes of multiple cancers. Ly6D mRNA expression was significantly higher in superficial bladder cancer (n=179) than infiltrating bladder cancer (n=175) in Sanchez-Carbayo [17], Stransky [44] and Lee [45] studies. Ly6D mRNA expression was significantly higher in medulloblastoma (n=60) than rhabdoid tumor (n=5) Pomeroy [46] study. Ly6D mRNA expression was significantly increased in triple negative breast cancer (TNBC) (n=700) compared to 2667 sample of non-TNBC (n=2667), grade 3 (n=47) than grade 2 (n=27), grade N1 (n=190) than grade N0 (n=137), tumors with p53 mutation (n=130) than p53 wildtype tumors (n=261), tumors with BRCA1 mutation (n= 38) than BRCA1 wildtype tumors (n=157), ERBB2 positive (n=92) than ERBB2 negative (n=48) tumors, basal type (n=16) than luminal (n=27) in The Cancer Genome Atlas (TCGA) (Unpublished, NCI), Stickeler [47], Minn [48], Waddell [49], Gluck [50], Bild [51], Kao [52], Bittner (unpublished, GSE2109), Farmer [53], Korde [54], Richardson [55], Esserman [56], Chin [57], Ginestier [58], vantVeer [59], Curtis [60], Ivshina [61], Bonnefoi [62] and Hatzis [63] studies. Ly6D mRNA expression was significantly upregulated in microsatellite instability in gastric and colorectal cancer as seen by D'Errico [64] and Jorissen [65] studies. High Ly6D mRNA expression was also correlated in more aggressive subsets of cervical cancer, esophageal and kidney cancer in Bittner (unpublished, GSE2109), Pyeon [66], TCGA [67], Kimchi [68] studies. Interestingly, in case of breast cancer depicted in Table 2, 14 studies show that Ly6D is significantly increased in TNBC while only one study show it is higher in ERBB2+ cancer compared to ERBB2- tumors. This suggests that while Ly6D is predominantly associated with TNBC tumor.

These results show that Ly6D expression was significantly increased in subtypes of bladder, brain and CNS, breast, pancreatic, gastric, cervical, colorectal, esophageal and kidney cancer.

High Ly6D expression and survival outcome in multiple cancers

Table 2 also shows that high Ly6D mRNA expression in brain cancer was significantly correlated with decreased one-year survival (dead, n=22 vs alive, n=100) in Pomeroy [46] and Phillips [69] studies. High Ly6D mRNA expression in pancreatic cancer was significantly correlated with decreased three-year survival (dead, n=19 vs alive, n=5) in Collisson [70] study. High Ly6D mRNA expression in breast cancer was significantly correlated with decreased three-year metastasis free survival (metastasis, n=79 vs metastasis free, n=85), decreased five-year metastasis free survival (metastasis, n=172 vs metastasis free, n=14), decreased three-year survival (dead, n=73 vs alive, n=496) decreased five-year

Table 1: Ly6D mRNA expression in normal and tumor tissue (n=number of samples) of multiple cancer types

Type of cancer	Reference	N (Normal)	N (Cancer)	Fold change	P-value
Bladder	[17]	48	28 (Superficial)	39.50	3.5E-12
			81 (Infiltrating)	6.63	2.6E-06
	[18]	9	28 (Superficial)	3.30	5.5E-04
			13 (Infiltrating)	3.17	8.0E-03
Brain and CNS	[19]	23	50 (Oligodendroglia)	1.27	1.3E-02
			81 (Glioblastoma)	1.20	3.6E-02
Breast	[20]	144	32 (Medullary)	1.58	2.0E-03
			1556 (Invasive ductal)	1.03	5.0E-03
	[21]	9	9 (Cancer)	1.21	1.5E-02
Head and neck	[22]	26 (Tongue)	31 (Tongue squamous)	7.56	4.5E-05
	[23]	9 (Thyroid)	9 (Thyroid papillary)	1.23	4.0E-03
	[24]	6 (Salivary)	16 (Salivary adenoid)	4.56	2.6E-02
Gastric	[25]	19	31 (Adeno)	1.25	8.0E-03
Lung	[26]	49	58 (Adeno)	1.43	5.1E-04
	[27]	58	58 (Adeno)	1.11	8.0E-03
	[28]	30	27 (Adeno)	2.29	1.0E-03
	[29]	20	226 (Adeno)	3.52	2.2E-07
	[30]	17	21 (Squamous)	12.71	2.0E-03
	[31]	65	27 (Squamous)	7.18	2.7E-06
	[32]	5	5 (Squamous)	3.28	3.7E-02
Ovarian	[33]	4	28 (Papillary)	1.07	1.0E-06
	[34]	4	8 (Clear cell adeno)	1.37	2.7E-02
	[35]	10	185 (Cancer)	1.17	8.4E-04
Pancreatic	[36]	16	36 (Cancer)	3.98	2.7E-06
	[37]	39	39 (Ductal adeno)	1.50	2.9E-02
Colorectal	TCGA	19	29 (Colon Mucinary adeno)	5.88	2.2E-07
		3	24 (Cecum adeno)	3.35	3.2E-07
		3	6 (Rectal Mucinary adeno)	3.22	1.1E-02
		3	60 (Rectal adeno)	2.78	9.4E-08
		19	102 (Colon adeno)	2.15	2.4E-12
	[38]	9	25 (Colon adeno)	2.25	4.0E-03
	[39]	5	13 (Colon Mucinary adeno)	1.62	5.0E-03
	[40]	65	65 (Rectal adeno)	1.57	1.9E-07
	[41]	24	45 (Colon adeno)	1.33	5.2E-04
Kidney	[42]	23	8 (Cancer)	4.22	2.9E-02
	[43]	5	19 (Papillary)	2.49	3.0E-03
			26 (Clear cell)	1.92	1.2E-02

Ly6D mRNA expression was significantly increased in bladder, brain and CNS, breast, head and neck, gastric, lung, ovarian, pancreatic, colorectal, and kidney cancer than their normal counterpart. Data observed using Oncomine (Invitrogen). N=number of patient samples.

Table 2: Ly6D mRNA expression in subset of multiple cancers

Type of cancer	Reference	Cancer		Fold change	P-value
		N (Group 1)	N (Group 2)		
Bladder	[17]	81 (Infiltrating)	28 (Superficial cancer)	5.96	7.6E-05
	[44]	32 (Infiltrating)	25 (Superficial cancer)	5.96	7.6E-05
	[45]	62 (Infiltrating)	126 (Superficial cancer)	1.75	8.0E-03
Brain and CNS	[46]	5 (Rhabdoid Tumor)	60 (Medulloblastoma)	24.87	4.0E-03
		40 (Medullo Alive at 1 year)	6 (Dead at 1 year)	3.18	5.9E-04
	[69]	60 (Astrocytoma Alive at 1 year)	16 (Dead at 1 year)	1.27	3.7E-01
Pancreatic	[70]	5 (Alive at 3 year))	19 (Dead at 3 year)	1.46	9.0E-03
Breast	TCGA	250 (Non-TNBC)	46 (TNBC)	5.42	3.1E-12
	[47]	24 (Non-TNBC)	8 (TNBC)	8.92	1.2E-02
		15 (Grade 2)	16 (Grade 3)	4.08	3.2E-02
	[48]	69 (Metastasis free 3 year)	12 (Metastasis at 3 year)	2.91	3.5E-02
		71 (Non-TNBC)	25 (TNBC)	2.04	1.2E-02
	[49]	44 (Non-TNBC)	22 (TNBC)	3.36	8.4E-04
		60 (BRCA1 wildtype)	20 (BRCA1 Mutation)	2.02	2.5E-02
	[50]	101 (Non-TNBC)	50 (TNBC)	3.22	1.0E-08
		72 (TP53 wildtype)	72 (TP53 Mutation)	1.63	6.0E-03
	[51]	124 (Alive at 3 year)	27 (Dead at 3 year)	1.90	3.8E-02
		60 (Alive at 5 year)	42 (Dead at 5 year)	1.62	3.4E-02
		48 (ERBB2 neg)	92 (ERBB2 pos)	1.51	2.0E-02
	[52]	295 (Non-TNBC)	32 (TNBC)	3.01	9.7E-04
		16 (Metastasis free 3 year)	67 (Metatasis at 3 year)	1.99	3.9E-02
		295 (Alive at 3 year)	31(Dead at 3 year)	2.74	9.7E-04
		137 (Grade N0)	190 (Grade N1+)	1.48	6.0E-03
	[71]	14 (Metastasis free 5 year)	172 (Metastasis at 5 year)	2.91	1.6E-04
	GSE2109	129 (Non-TNBC)	39 (TNBC)	1.79	2.4E-02
	[53]	27 (Luminal)	16 (Basal)	2.47	1.0E-03
	[54]	39 (Non-TNBC)	21 (TNBC)	2.28	3.9E-04
	[55]	19 (Non-TNBC)	18 (TNBC)	2.19	1.2E-02
	[56]	74 (Non-TNBC)	24 (TNBC)	2.10	4.8E-04
		77 (Alive at 3 year)	15(Dead at 3 year)	2.74	9.7E-04
	[57]	87 (Non-TNBC)	19 (TNBC)	1.88	6.0E-03
	[58]	12 (Grade 2)	31 (Grade 3)	1.81	1.6E-02
	[59]	97 (BRCA1 wildtype)	18 (BRCA1 Mutation)	1.64	6.7E-04
	[60]	1340 (Non-TNBC)	211 (TNBC)	1.43	2.0E-03
	[61]	189 (TP53 Wildtype)	58 (TP53 (Mutation)	1.49	3.4E-04
	[62]	32 (Non-TNBC)	80 (TNBC)	1.43	8.6E-06
	[63]	320 (Non-TNBC)	178 (TNBC)	1.41	7.6E-09
Gastric	[64]	12 (Microsatellite stable)	14 (Microsatellite Instable)	3.94	6.0E-03
Cervical	GSE2109	9 (Adeno)	23 (Squamous)	12.05	2.0E-03
	[66]	15 (Stage M0)	4 (Stage M1+)	3.20	1.1E-02
Colorectal	TCGA [67]	24 (Cecum adeno)	20 (Colon Muc adeno)	1.89	6.0E-03
	[65]	16 (rectal adeno)	137 (Colon adeno)	1.75	1.2E-02
		77 (Microsatellite stable)	78 (Microsatellite unstable)	1.45	2.9E-02
	[72]	15 (Alive at 5 year)	20 (Dead at 5 year)	1.42	3.9E-02
Esophageal	[68]	8 (Precursor)	8 (Cancer)	46.55	4.0E-03
Kidney	TCGA [67]	72 (Clear cell)	16 (Papillary)	4.12	4.0E-03
	GSE2109	10 (Grade 2)	6 (Grade 3)	2.02	1.5E-02

Ly6D mRNA expression was significantly increased in subtypes of bladder, brain and CNS, pancreatic, breast, gastric, cervical, colorectal, esophageal and kidney cancer. High Ly6D expression was significantly correlated with poor clinical outcome in brain and CNS, pancreatic, and colorectal cancer. Data observed using Oncomine (Invitrogen). N=number of patient samples.

survival (dead, n=42 vs alive, n=60) in Minn [48], Bild [51], Kao [52], Bos [71], and Essermann [56] studies.

High Ly6D mRNA expression in breast cancer was significantly correlated with poor outcome in five-year distant metastasis free survival (low Ly6D, n=818; high Ly6D, n=790; HR=1.29, *p*=0.012, n= number of patient, HR=hazard ratio), post progression free survival (low Ly6D, n=231; high Ly6D, n=120; HR=1.57, *p*=9.0E-04), relapse free survival (low Ly6D, n=1133; high Ly6D, n=2421; HR=1.30, *p*=2.0E-05) shown by KM plotter and five-year relapse free survival (low Ly6D, n=57; high Ly6D, n=57; HR=1.48, *p*=0.006) shown by PROGgeneV2 (Table S1, Figure 1A).

High Ly6D mRNA expression in colon cancer was significantly correlated with poor outcome in relapse free survival (low Ly6D, n=25; high Ly6D, n=26; HR=1.19, *p*=0.0469) and overall survival (low Ly6D, n=25; high Ly6D, n=26; HR=1.63, *p*=0.0199) shown by PROGgeneV2 (Table S1) and overall survival in 5-year overall survival (low Ly6D, n=15; high Ly6D, n=20, *p*=3.9E-02) in Smith study [72] (Table 2).

High Ly6D mRNA expression in lung cancer was significantly correlated with poor outcome in five-year overall survival with no restriction (low Ly6D, n=1323; high Ly6D, n=603; HR=1.49, *p*=1.70E-09) or with restriction of lung adenocarcinoma (low Ly6D, n=538; high Ly6D, n=181; HR=2.11, *p*=7.60E-10), first progression free survival with no restriction (low Ly6D, n=712; high Ly6D, n=270; HR=1.33, *p*=0.006) or with restriction of lung adenocarcinoma (low Ly6D, n=345; high Ly6D, n=116; HR=1.71, *p*=0.001) and post progression free survival with restriction of lung adenocarcinoma (low Ly6D, n=257; high Ly6D, n=87; HR=1.48, *p*=0.006), by KM plotter and five-year relapse free survival with restriction of lung adenocarcinoma (low Ly6D, n=112; high Ly6D, n=113; HR=1.38, *p*=4.00E-04) shown by PROGgeneV2 (Table S1, Figure 1B).

High Ly6D mRNA expression in gastric cancer was significantly correlated with poor outcome in five-year post progression free survival (low Ly6D, n=209; high Ly6D, n=432; HR=1.38, *p*=0.0047) shown by KM plotter (Table S1, Figure 1C).
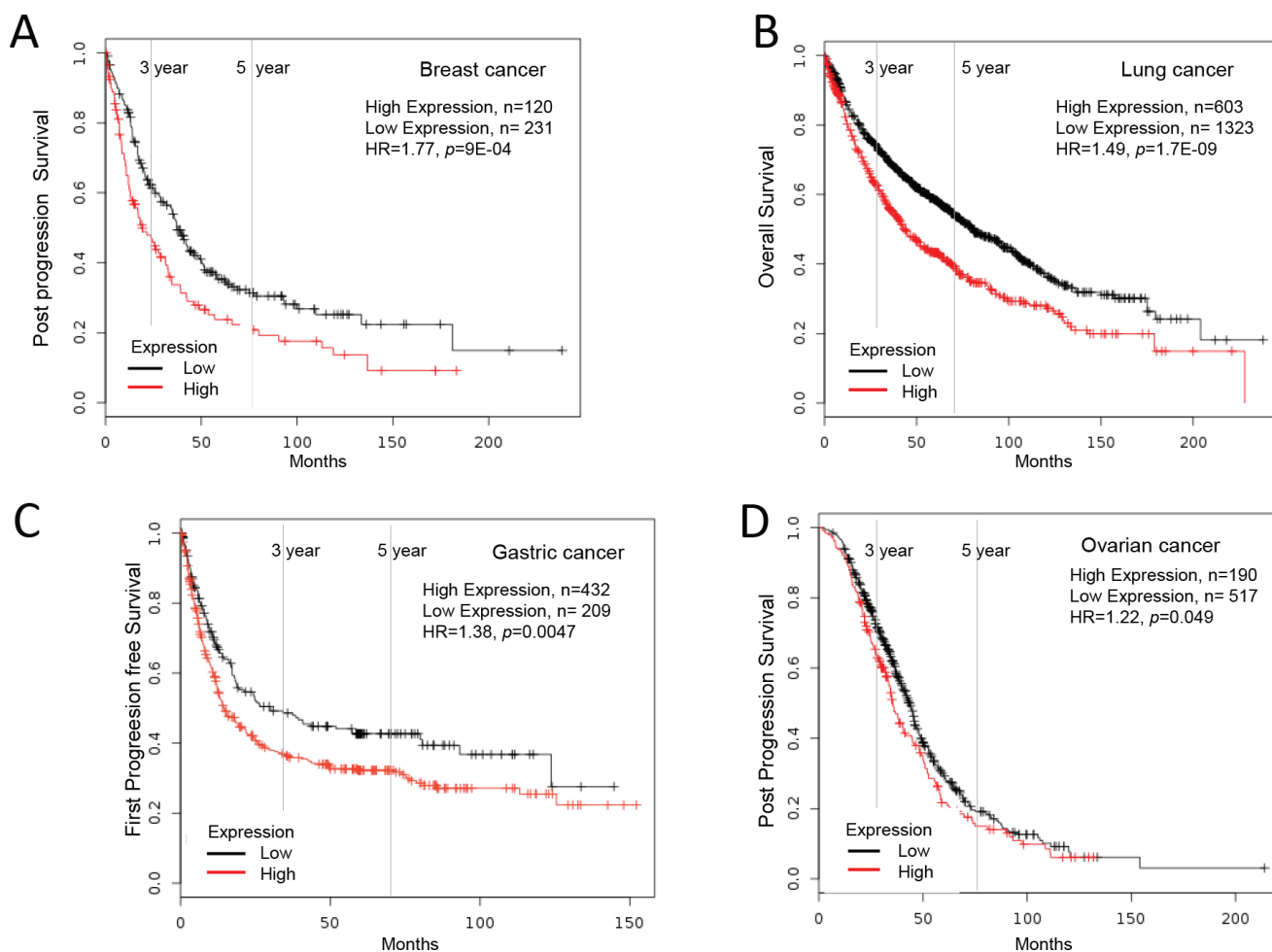

Figure 1: Increased Ly6D mRNA expression in cancer and patient survival. High Ly6D expression leads to poor survival in **A.** breast cancer, **B.** lung cancer, **C.** gastric cancer and **D.** ovarian cancer.

High Ly6D mRNA expression in ovarian cancer was significantly correlated with poor outcome in five-year post progression free survival (low Ly6D, n=517; high Ly6D, n=190; HR=1.22, p=0.049) shown by KM plotter (Table S1, Figure 1D).

These data show that high Ly6D expression was significantly correlated with poor clinical outcome in brain and CNS, pancreatic, and colorectal, breast, colorectal, lung, gastric and ovarian cancer.

Increased expression of Ly6E in multiple cancers

To examine the status of Ly6E in human cancer, we used Oncomine or G-DOC to analyze gene expression omnibus (GEO) datasets. The data in Table 3A shows a significant increased expression of Ly6E in bladder cancer (n=150) than normal tissues (n=57) in Sanchez-Carbayo [17] and Dryskjot [18] studies. Ly6E mRNA expression was significantly increased in breast cancer (n=2613) than normal tissues (n=235) in TCGA, Radvani [73], Curtis [20], Ma [74], Gluck [75], and Zhao [76] studies. Ly6E mRNA expression was significantly increased in esophageal cancer (n=78) than normal tissues (n=78) in Kimchi [68], Hu [77] and Su [78] studies. Ly6E mRNA expression was significantly increased in gastric cancer (n=89) than normal tissues (n=62) in D'Errico [64], Cho [25] and Wang [79] studies. Ly6E mRNA expression was significantly increased in pancreatic cancer (n=85) than normal tissues (n=60) in Logsdon [80], Badea [37] and Pei [81] studies. Ly6E mRNA expression was significantly increased in cervical cancer (n=90) than normal tissues (n=34) in Scotto [82], Pyeon [66] and Biewenga [83] studies. Ly6E mRNA expression was significantly increased in colorectal cancer (n=10) than normal tissues (n=5) in Skrzypczak [84] study. Ly6E mRNA expression was significantly increased in prostate cancer (n=36) than normal tissues (n=17) in Tomlins [85] study. The data in Table 3B shows a significantly increased Ly6E mRNA expression in lung cancer (n=514) than normal tissues (n=220) in Okayama [29], Talbot [86], Beer [87], Su [28], Wei [88], Selamat [27] and Landi [26] studies. Ly6E mRNA expression was significantly increased in head and neck cancer (n=396) than normal tissues (n=17) Toruner [89], Giordano [90], Ye [91], Peng [92], Peng 2 [92], He [23], Cromer [93], Estilo [22], Vasko [94], Ginos [95] and Frierson [24] studies. Ly6E mRNA expression was significantly increased in ovarian cancer (n=396) than normal tissues (n=40) in Yoshihara [96], Adib [97], TCGA (NCI, unpublished), Welsh [98], Bonome [99] and Henedrix [34] studies. Ly6E mRNA expression was significantly increased in kidney cancer (n=155) than normal tissues (n=68) in Yusenko [43], Beroukhim [100], Jones [42], Cutcliffe [101], Gumz [102], and Lenburg [103] studies. Ly6E mRNA expression was significantly increased in melanoma (n=45) than normal skin (n=7) in Talantov [104] study. Ly6E mRNA expression was

significantly increased in embryonic tumors (n=24) than normal testis (n=6) in Korkola [105] study and pleural malignant mesothelioma (n=40) than normal samples (n=9) of pleura in Gordon [106] study.

These results show that Ly6E expression was significantly increased in bladder, breast, esophageal, gastric, pancreatic, cervical, colorectal, prostate, lung, head and neck, ovarian, kidney, melanoma, embryonic cancer than their counterpart normal tissues.

The data in Table 4 shows that Ly6E mRNA expression was significantly increased in subtypes of multiple cancers. Ly6E mRNA expression was significantly higher in superficial bladder cancer (n=28) than infiltrating bladder cancer (n=81) and high expression of Ly6E was correlated with higher grade (infiltrating grade 3, n=75 vs infiltrating grade 2, n=6) in Sanchez-Carbayo [17] study. Ly6E mRNA expression was significantly higher in medulloblastoma with CTNNB1 mutation (n=8) than CTNNB1 wildtype tumors (n=38), medulloblastoma with CTNNB1 positive by immunohistochemistry (IHC) (n=6) than CTNNB1 IHC negative (n=44), tumors with MycN amplification (n=14) than tumors not amplified for MycN (n=32) in Kool [107], Robinson [108] and Janoueix-Lerosey [109] studies. Ly6E mRNA expression was significantly higher in esophageal cancer (n=83) than precursor (n=23) in Kimchi [68] and Su [78] studies. Ly6E mRNA expression was significantly higher in pancreatic cancer (n=10) than precursor (n=5) in Logsdon [80] study. High expression of Ly6E was correlated with higher grade of breast cancer. Ly6E mRNA expression was significantly high in ductal N1+ stage (n= 222) than ductal N0 stage (n=274) in Bittner (unpublished, GSE2109), Julka [110], and Ivshina [61], studies and grade 3 tumor (n=334) than grade 1 (n=334) in Loi [111], Buffa [112], Miller [113] and Sotiriou [114] studies, grade 3 (n=64) than grade 2 (n=34) in Bonnefoi [62] study, invasive ductal (n=31) than non-invasive ductal (n=3) in Radvanyi [73] study. Ly6E mRNA expression was significantly higher in tumors with TP53 mutaions (n=130) than tumors with wildtype tumor (n=261) and in tumors with BRCA1 mutaions (n=31) than tumors with BRCA1 wildtype (n=128) in Ivshina [61], Gluck [50], Pawitan [115] studies. (Ly6E) mRNA was found significantly increased in TNBC (n=286) than non-TNBC (n=1653) in Curtis [60], TCGA (Unpublished, NCI), Stickeler [47] and Korde [54] studies.

These results show that Ly6E expression was significantly increased in subtypes of bladder, brain, esophageal, pancreatic and breast cancer.

High Ly6E expression and survival outcome in multiple cancers

Table 4 also shows a high Ly6E mRNA expression in bladder cancer was significantly correlated with decreased three-year survival (dead, n=33 vs alive, n=19) in Lee [45] study.

Table 3: Ly6E mRNA expression in normal and tumor tissue of multiple cancer types

A. Ly6E is significantly increased in bladder, breast, Esophageal, gastric, pancreatic, cervical, colorectal and prostate cancer than their normal counterparts

Type of cancer	Reference	N (Normal)	N (Cancer)	Fold change	P-value
Bladder	[17]	48	28 (Superficial)	4.64	4.3E-12
			81 (Infiltrating)	3.09	2.1E-08
	[18]	9	13 (Infiltrating)	2.09	4.7E-04
			28 (Syperficial)	1.70	1.8E-04
Breast	TCGA	61	3 (Adeno)	1.42	1.5E-02
			76 (Invasive)	1.82	2.4E-08
			389(Invasive Ductal)	1.47	1.7E-06
			36 (Invasive Lobular)	1.32	1.3E-02
	[73]	9	3 (Ductal)	3.25	3.0E-03
			3 (Invasive Medulary)	2.17	1.5E-02
	[20]	144	32 (Medullary)	2.10	2.9E-05
			1556 (Invasive Ductal)	1.55	4.8E-21
			46 (Mucinous)	1.65	3.0E-05
			10 (Ductal)	1.42	2.1E-02
			21(Invasive)	1.35	1.6E-02
			148 (Invasive Lobular)	1.15	8.0E-03
			90 (Invasive Lobular & Ductal)	1.13	4.9E-02
	[74]	14	9 (Ductal)	1.18	4.0E-03
	[75]	4	154 (Invasive)	1.19	6.0E-03
	[76]	3	37 (Invasive Ductal)	1.91	3.6E-02
Esophageal	[68]	8	8 (Adeno)	2.21	2.4E-02
	[77]	17	17 (Squamous)	3.00	4.3E-05
	[78]	53	53 (Squamous)	1.78	7.7E-13
Gastric	[64]	31	26 (Intestinal Adeno)	9.65	2.3E-12
	[25]	19	31 (Diffuse Aden)	3.40	3.5E-07
			20 (Intestinal Adeno)	2.94	2.9E-04
	[79]	12	12 (Cancer)	2.46	5.0E-03
Pancreatic	[80]	5	10 (Adeno)	3.21	5.3E-04
	[37]	39	39 (PD adeno)	3.05	5.2E-16
	[81]	16	36 (Tumor)	3.49	2.1E-07
Cervical	[82]	21	32 (Squamous)	2.08	4.1E-04
	[66]	8	20 (Cancer)	1.56	7.0E-03
	[83]	5	40 (Squamous)	1.37	1.2E-02
Colorectal	[84]	10	5 (Adeno carcinoma)	1.84	8.1E-06
			5 (Cancer)	2.59	1.8E-05
Prostate	[85]	17	24 (Cancer)	1.72	6.0E-03
			12 (Prostatic Intraepithelial)	1.69	6.0E-03

B. Ly6E is significantly increased in lung, head and neck, ovarian, kidney, melanoma md embryonic tumors than their normal counterparts

Type of cancer	Reference	N (Normal)	N (Cancer)	Fold change	P-value
Lung	[29]	20	226 (Adeno)	2.31	8.6E-18
	[86]	28	34 (Squamous)	1.86	2.0E-09
	[87]	10	86 (Adeno)	1.40	4.0E-03
	[28]	30	27 (Adeno)	1.89	4.0E-03
	[88]	25	25 (Adeno)	1.55	8.6E-05
	[27]	58	58 (Adeno)	1.60	2.3E-05
	[26]	49	58 (Adeno)	1.24	9.0E-03
Head and neck	[89]	4(Squamous)	16 (Squamous)	3.43	3.1E-07
	[90]	4(thyroid)	26(Thyroid papillary)	1.74	6.6E-09
			10 (Tall ceil papillary)	1.67	3.0E-05
			4 (Thyroid anaplastic)	2.22	1.0E-03
	[91]	12 (Tongue)	26 (Tongue squamous)	1.27	5.8E-04
	[92]	22 (Oral cavity)	57 (Squamous)	2.22	2.0E-19
	[92]	10 (Oral cavity)	112 (Squamous)	1.12	1.1E-24
	[23]	9 (Thyroid)	9 (Thyroid papillary)	2.56	5.3E-05
	[93]	4 (Uvula)	34 (Squamous)	1.64	5.2E-04
	[22]	26 (Tongue)	31 (Tongue squamous)	3.67	1.9E-08
	[94]	4 (Thyroid)	14 (Thyroid papillary)	2.72	1.0E-03
	[95]	13 (Buccal mucosa)	41 (Squamous)	2.70	7.1E-07
	[24]	6 (Salivary)	16 (Salivary adenoid)	17.46	3.3E-02
Ovarian	[96]	10	43 (Serous adeno)	3.15	5.5E-08
	[97]	4	6 (Serous adeno)	2.31	7.0E-03
	TCGA	8	586 (Serous cyst adeno)	2.81	9.1E-05
	[98]	4	28 (Serous papillary)	1.25	1.6E-02
	[99]	10	85 (Cancer)	1.73	1.8E-04
	[34]	4	41 (Serous adeno)	1.27	1.2E-02
Kidney	[43]	5	26 (Clear cell)	4.08	1.4E-07
			4 (Wilms)	2.70	7.0E-03
	[100]	11	32 (Hereditary Clear cell)	4.37	1.1E-10
	[42]	23	8 (Urothelial Carcinoma)	3.00	2.9E-04
			23 (Clear cell)	2.26	2.5E-04
			11 (Papillary)	1.39	2.8E-02
	[101]	3	18 (Wilms)	2.39	5.0E-03
			14 (Clear cell Sarcoma)	1.68	3.3E-02
	[102]	10	10 (clear cell)	1.64	4.9E-02
	[103]	9	9 (Clear cell)	1.63	1.0E-03
Melanoma	[104]	7	45 (Cutaneous)	3.02	2.5E-06
Mixed	[105]	6 (Normal testis)	9 (Yolk Sac tumor)	5.32	2.7E-08
			15 (Embryonal)	4.74	1.2E-10
	[106]	9	40 (Pleural Malignant Mesothelioma)	4.45	7.8E-06

Data observed using Oncomine (Invitrogen) and G-DOC. N=number of patient samples

Table 4: Ly6E mRNA expression in subset of multiple cancers

Type of cancer	Reference	Cancer		Fold change	P-value
		N (Group 1)	N (Group 2)		
Bladder	[17]	6 (Infiltrating Grade2)	75 (Infiltrating Grade 3)	3.23	3.3E-02
		81 (Infiltrating)	28 (Superficial)	1.50	9.0E-03
	[45]	19 (Alive at 3 years)	33 (dead at 3 years)	1.72	3.9E-02
Brain and CNS	[108]	44 (Medulloblastoma CTNNB1 IHC neg)	6 (Medulloblastoma CTNNB1 IHC neg)	2.27	2.0E-03
	[107]	38 (Medulloblastoma CTNNB1 WT)	8 (Medulloblastoma CTNNB1 mutation)	3.11	5.9E-05
	[109]	32(No MycN amplification)	14 (MycN amplification)	1.93	1.1E-02
	[116]	56 (Neuroblastoma No recurrence 5 year)	46 (Neuroblastoma Recurrence 5 year)	1.63	9.4E-06
Esophageal	[68]	8 (Precursor)	8 (Cancer)	2.22	3.6E-02
	[78]	15 (Precursor)	75 (Cancer)	1.78	5.0E-03
Pancreatic	[80]	5 (Precursor)	10 (Cancer)	3.05	9.4E-06
Breast	GSE2109	94 (Ductal N0)	123 (Ductal N1+)	1.46	4.0E-03
	[110]	21 (Ductal N0)	18 (Ductal N1+)	1.46	1.7E-02
	[61]	159 (Ductal N0)	81 (Ductal N1+)	1.27	7.4E-04
	[111]	147(Grade 1)	136(Grade 3)	1.38	1.1E-02
	[112]	42(Grade 1)	65(Grade 3)	1.39	2.0E-02
	[111]	14(Grade 1)	24(Grade 3)	1.53	3.1E-02
	[113]	67(Grade 1)	54(Grade 3)	1.51	2.1E-05
	[114]	64(Grade 1)	55(Grade 3)	1.40	2.0E-03
	[62]	34 (Grade 2)	64 (Grade 3)	1.29	3.0E-02
	[73]	3 (Ductal)	31(Invasive Ductal)	2.25	1.3E-02
	[61]	189 (TP53 wildtype)	58 (TP53 Mutation)	1.30	7.7E-04
	[50]	72 (TP53 wildtype)	72 (TP53 Mutation)	1.25	1.0E-02
	[115]	128 (BRCA1 wildtype)	31 (BRCA1 Mutation)	1.24	1.5E-02
	[60]	1340 (non-TNBC)	211 (TNBC)	1.52	2.9E-21
	TCGA	250 (non-TNBC)	46 (TNBC)	1.55	2.0E-05
	[47]	24 (non-TNBC)	8 (TNBC)	5.28	5.0E-03
	[54]	39 (non-TNBC)	21 (TNBC)	1.51	1.1E-02
	[71]	148 (Metastasis free 1 year)	49 (Metastasis at 1 year)	1.34	2.4E-02
	[117]	171 (Metastasis free 3 year)	19 (Metastasis at 3 year)	1.63	4.0E-03
	[52]	32 (Metastasis free 3 year)	50 (Metastasis at 3 year)	1.64	3.0E-03
	[63]	223 (Metastasis free 3 year)	99 (Metastasis at 3 year)	1.25	4.0E-03
Gastric	[118]	7 (Metastasis free 5 years)	11 (Metastasis at 5 year)	2.32	1.4E-02
	[119]	34 (Alive at 1 year)	12 (Dead at 1 year)	1.25	5.0E-03

Ly6E mRNA expression was significantly increased in subtypes of bladder, brain and CNS, esophageal pancreatic, breast and gastric cancer. High Ly6E expression was significantly correlated with poor clinical outcome in bladder, brain and CNS, breast and gastric cancer. Data observed using Oncomine (Invitrogen) and G-DOC. N=number of patient samples.

High Ly6E mRNA expression in neuroblastoma was significantly correlated with five-year recurrence free survival (recurrence, n=46 vs no recurrence, n=56) in Asgharzadeh [116] study. High Ly6E mRNA expression in breast cancer was significantly correlated with decreased one-year metastasis free survival (metastasis, n=49 vs metastasis free, n=148), decreased three year metastasis free survival (metastasis, n=168 vs metastasis free, n=426) in Bos [71], Schmidt [117], Kao [52] and Hatzis [63] studies. High Ly6E mRNA expression in gastric cancer was significantly correlated with decreased five-year metastasis free survival (metastasis, n=11 vs metastasis free, n=7), decreased one-year overall survival (dead, n=12 vs alive, n=34) in Forster [118] and Chen [119] studies.

High Ly6E mRNA expression in glioma was significantly correlated with poor five-year overall survival (low Ly6E, n=14; high Ly6E, n=14; HR=2.34, *p*=0.0026, n= number of patient, HR=hazard ratio), shown by PROGgeneV2 (Table S2, Figure 2A). High Ly6E mRNA expression in breast cancer was significantly correlated with poor five-year overall survival with restriction of grade 1 breast cancer (low Ly6E, n=75; high Ly6E, n=60; HR=3.48, *p*=0.022) or without restriction (low Ly6E, n=646; high Ly6E, n=653; HR=1.4, *p*=0.007), five-year relapse free survival with restriction of grade 1 breast cancer (low Ly6E, n=179; high Ly6E, n=129; HR=2.52, *p*=0.001), grade 2 breast cancer (low Ly6E, n=388; high Ly6E, n=336; HR=1.53, *p*=0.001), or without restriction (low Ly6E, n=1113; high Ly6E, n=2441; HR=1.32, *p*=1.50E-05), five-year distant metastasis free survival (low Ly6E, n=479; high Ly6E, n=1130; HR=1.71, *p*=1.90E-05), five-year post progression free survival (low Ly6E, n=99; high Ly6E, n=252; HR=1.43, *p*=0.018), shown by KM plotter. High Ly6E mRNA expression in breast cancer was significantly correlated with poor five-year overall survival with restriction of estrogen receptor positive breast cancer (low Ly6E, n=112; high Ly6E, n=113; HR=1.39, *p*=0.002), with restriction of progesterone receptor positive breast cancer (low Ly6E, n=112; high Ly6E, n=113; HR=1.39, *p*=0.002) or without restriction (low Ly6E, n=15; high Ly6E, n=16; HR=2.73, *p*=0.02), and five-year distance metastasis free survival (low Ly6E, n=112; high Ly6E, n=113; HR=1.44, *p*=0.000) shown by PROGgeneV2 (Table S2, Figure 2B).

High Ly6E mRNA expression in gastric was significantly correlated with poor five-year overall survival (low Ly6E, n=314; high Ly6E, n=562; HR=2.08, *p*=1.3E-14) shown by KM plotter (Table S2, Figure 2C).

High Ly6E mRNA expression in lung cancer was significantly correlated with poor five-year overall survival with restriction of stage IIIa cancer (low Ly6E, n=14; high Ly6E, n=14; HR=6.82, *p*=0.0273) and poor five-year relapse free survival with restriction of stage IIb cancer (low Ly6E, n=22; high Ly6E, n=23; HR=1.97, *p*=0.029) or with restriction of stage 1b cancer (low Ly6E, n=27; high

Ly6E, n=27; HR=1.7, *p*=0.046) shown by PROGgeneV2 (Table S2, Figure 2D).

High Ly6E mRNA expression in ovarian cancer was significantly correlated with poor five-year overall survival with restriction of Stage4, serous, grade 3 cancer (low Ly6E, n=52; high Ly6E, n=51; HR=1.7, *p*=0.036) shown by KM plotter (Table S2, Figure 2E).

High Ly6E mRNA expression in colorectal cancer was significantly correlated with poor five-year relapse free survival with restriction of age greater than 50 years (low Ly6E, n=82; high Ly6E, n=83; HR=1.70, *p*=0.018) or without any restriction (low Ly6E, n=93; high Ly6E, n=94; HR=1.77, *p*=0.0005) shown by PROGgeneV2 (Table S2, Figure 2F).

These data show that high Ly6E expression was significantly correlated with poor clinical outcome in glioma, breast, gastric, lung, ovarian and colorectal cancer.

Increased expression of Ly6H in multiple cancers

To examine the status of Ly6H in human cancer, we used Oncomine or G-DOC to analyze gene expression omnibus (GEO) datasets. As shown in Table 5 we found a significant increased expression of Ly6H in brain and CNS cancer (n=27) than normal relevant tissue (n=10) in Shai [120] and Lee [121] studies. Ly6H mRNA expression was significantly increased in esophageal cancer (n=104) than normal tissue (n=42) in Hao [122] and Kim [123] studies. Ly6H mRNA expression was significantly increased in breast cancer (n=2567) than normal tissue (n=209) in TCGA (unpublished, NCI), Curtis study [20] and Gluck [50] studies. Ly6H mRNA expression was significantly increased in Kidney cancer (n=22) than normal tissue (n=8) in Yusenko [43] and Cutcliffe [101] studies. Ly6H mRNA expression was increased significantly in head and neck cancer (n=20) than normal tissue (n=9) in Schlingemann [124] and Frierson [24] studies. Ly6H mRNA expression was significantly increased in lung cancer (n=47) than normal tissues (n=47) in Bhattacharjee [30] and Su [28] studies. Ly6H mRNA expression was significantly increased in ovarian cancer (n=291) than normal tissues (n=19) in Bonome [35] Lu [125] and Hendrix [34] studies.

These results show that Ly6H expression was significantly increased in brain and CNS, esophageal, breast, kidney, head and neck, lung and ovarian cancer than their counterpart normal tissues.

As shown in Table 6, we found that Ly6H mRNA expression was significantly increased in subtypes of multiple cancers. Ly6H mRNA expression was significantly higher in Myc amplified brain and CNS cancer (n= 50) than cancer without Myc amplification (n= 102) in Wang [79] and Robinson [108] studies. Ly6H mRNA expression was significantly higher in estrogen receptor (ER) positive breast cancer (n=562) than ER negative breast cancer (n=244) in Bittner (Unpublished, GSE2109), Wang [126], Stickeler [47] and TCGA
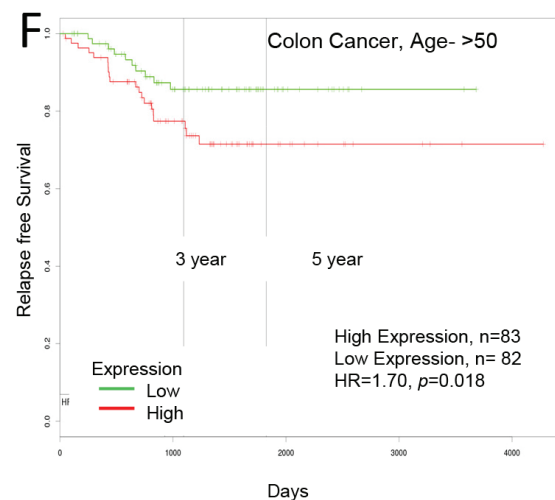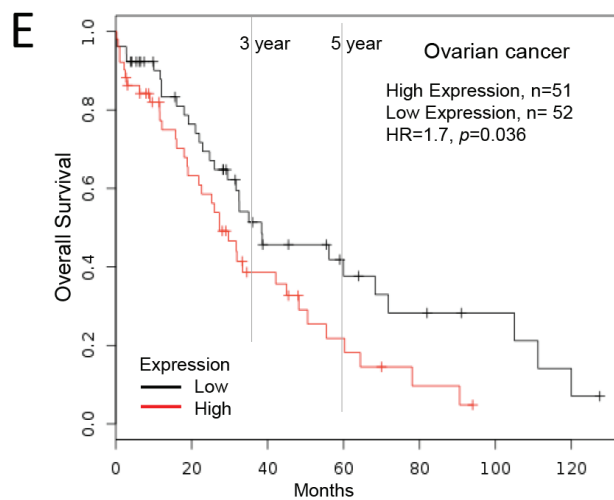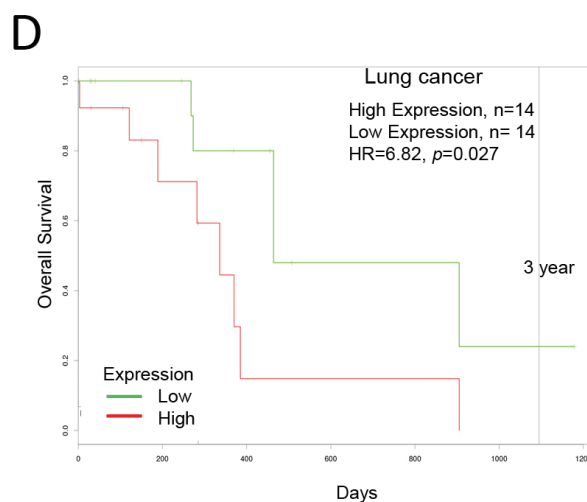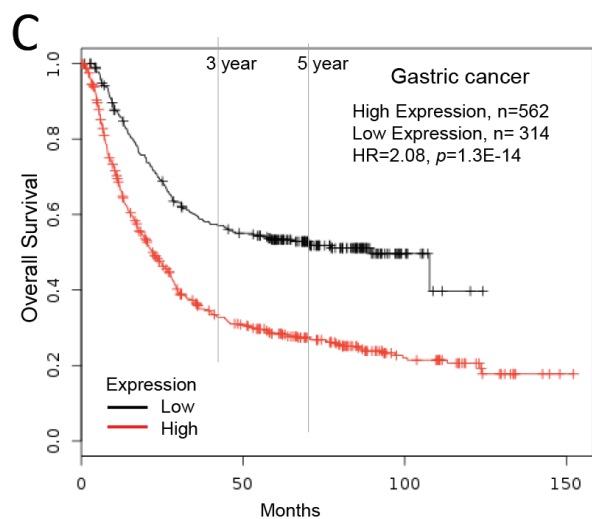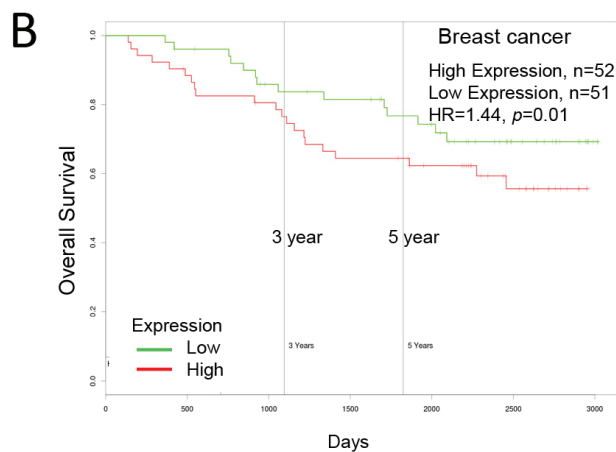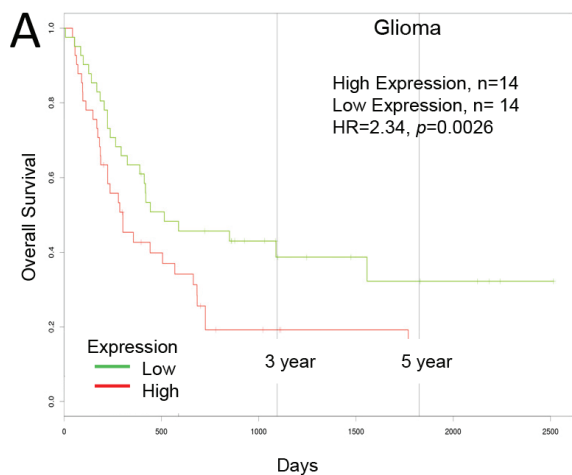
Figure 2: Increased Ly6E expression in cancer and patient survival. High Ly6E expression leads to poor survival in **A.** glioma, **B.** breast cancer, **C.** gastric cancer, **D.** lung cancer, **E.** ovarian cancer and **F.** colorectal cancer.

Table 5: Ly6H mRNA expression in normal and tumor tissue of multiple cancer types

Type of cancer	Reference	N (Normal)	N (Cancer)	Fold change	P-value
Brain and CNS	[120]	7 (White matter)	5 (Astrocytoma)	2.66	7.0E-03
	[121]	3 (Neural stem)	22 (Glioblastoma)	1.93	3.8E-02
Esophageal	[122]	14	14 (Barrett's)	1.73	9.9E-04
	[123]	28	15 (Barrett's)	1.38	6.5E-06
			75 (Adeno)	1.29	4.3E-08
Breast	TCGA	61	36 (Invasive Lobular)	2.72	3.6E-15
			389 (Invasive Ductal)	2.10	6.4E-24
			3 (Male)	3.80	2.5E-02
			4 (Mucinous)	6.18	2.2E-02
			3 (Invasive Ductal and Lobular)	3.10	4.6E-02
			7 (Ductal and Lobular)	2.35	3.0E-03
	[20]	144	10 (Ductal)	1.24	1.2E-02
			46 (Mucinous)	1.61	1.4E-09
			32 (Medullary)	1.20	2.0E-04
			1556 (Invasive Ductal)	1.35	1.0E-55
			148 (Invasive Lobular)	1.37	2.3E-19
			90 (Invasive Ductal and Lobular)	1.34	7.9E-18
			3 (Benign)	1.08	3.0E-02
			14 (Cancer)	1.18	4.6E-04
			5 (Phyllodes)	2.08	1.6E-02
			67 (Tubular)	1.36	7.0E-13
	[50]	4	154 (Invasive)	2.80	5.0E-03
Kidney	[43]	5	4 (Wilms)	3.26	1.7E-02
	[101]	3	18 (Wilm)	1.37	1.0E-03
Head and Neck	[124]	3 (Hypopharynx)	4 (Squamous)	1.23	3.9E-02
	[24]	6 (Salivary)	16 (Adenoid cystic)	9.13	2.8E-02
Lung	[30]	17	20 (Carcinoid)	14.05	3.2E-07
	[28]	30	27 (Adeno)	1.37	2.2E-02
Ovarian	[35]	10	185 (Cancer)	1.20	4.0E-07
	[125]	5	7 (Clear cell adeno)	1.19	1.9E-02
	[34]	4	13 (Mucinous adeno)	1.23	1.2E-02
			8 (Clear cell adeno)	1.17	3.1E-02
			37 (Endometrioid adeno)	1.18	2.6E-02
			41 (Serous adeno)	1.15	4.4E-02

Ly6H is significantly increased in brain and CNS, esophageal, breast, kidney, head and neck, lung and ovarian cancer than their normal counterparts. Data observed using Oncomine (Invitrogen). N=number of patient samples.

Table 6: Ly6H mRNA expression in subset of multiple cancers

| Type of cancer | Reference | Cancer | | Fold change | P-value |
		N (Group 1)	N (Group 2)		
Brain and CNS	[79]	81 (Myc not amplified)	20 (Myc amplified)	2.64	9.1E-04
	[108]	21 (Myc not amplified)	30 (Myc amplified)	1.52	1.0E-02
Breast	GSE2109	66 (ER neg)	110 (ER pos)	1.24	2.3E-02
	[126]	77 (ER neg)	209 (ER pos)	1.27	2.0E-03
	[47]	14 (ER neg)	18 (ER pos)	2.19	9.0E-03
		15 (PgR neg)	17 (PgR pos)	2.17	1.0E-02
	TCGA	87 (ER neg)	225 (ER pos)	1.31	4.1E-04
		127 (PgR neg)	189 (PgR pos)	1.27	3.7E-04
	[127]	7 (PgR neg)	9 (PgR pos)	2.41	4.6E-02
	[129]	40 (Grade 1)	26 (Grade 2)	1.21	6.0E-03
	[73]	30 (Ductal)	5 (Lobular)	2.35	1.0E-03
	[128]	158 (Ductal)	17 (Lobular)	1.28	2.6E-02
		105 (Recurrence free at 5 year)	47 (Recurrence at 5 year)	1.25	1.0E-02
		123 (Metastasis free at 5 year)	29 (Metastasis at 5 year)	1.23	1.0E-02
Gastric	[25]	4 (Stage 1)	14 (Stage 3)	1.56	3.0E-03
Kidney	GSE2109	43 (Others)	184 (Clear cell)	1.43	4.0E-03
	TCGA	16 (Papillary)	72 (Clear cell)	1.68	4.3E-05
Pancreatic	[140]	23 (Precursor)	8 (Cancer)	1.22	2.0E-02
Cervical	[130]	7 (Precursor)	21 (Cancer)	1.37	5.0E-03
Colorectal	[131]	43 (KRAS wildtype)	27 (KRAS Mutation)	1.64	4.0E-03
	[65]	30 (Recurrence free at 5 year)	56 (Recurrence at 5 year)	1.24	3.3E-02

Ly6H mRNA expression was significantly increased in subtypes of brain and CNS, breast, gastric, kidney, pancreatic, cervical and colorectal cancer. High Ly6H expression was significantly correlated with poor clinical outcome in breast and colorectal cancer. Data observed using Oncomine (Invitrogen). N=number of patient samples.

(Unpublished, NCI) studies. Ly6H mRNA expression was significantly higher in progesterone receptor (PgR) positive breast cancer (n=215) than PgR negative breast cancer (n=149) in Stickeler [47] and TCGA (Unpublished, NCI) and Chang [127] studies. Ly6H mRNA expression was significantly higher in lobular breast cancer (n=22) than ductal breast cancer (n=188) in Radvanyi [73] and Desmedt [128] studies. Increased Ly6H mRNA expression was significantly correlated with more aggressive phenotype of breast cancer - grade 2 (n=40) than grade 1 (n=40), gastric cancer - stage 3 (n=14) than stage 1 (n=4) in Curtis [129], and Cho [25] studies. Ly6H mRNA expression was significantly increased in clear cell carcinoma of kidney (n=256) than other types (n=59) in TCGA [67] and Bittner(Unpublished, GSE2109) studies.

Ly6H mRNA expression was significantly increased in cancerous tissue of cervix (n=8) than precursors cervix cancer (n=23) in Zhai [130] study. Ly6H mRNA expression was significantly increased in colorectal cancer with KRAS mutation (n=27) than KRAS wildtype tumors (n=43) in Khambata-Ford [131] study.

These results show that Ly6H expression was significantly increased in subtypes of brain and CNS, breast, gastric, kidney, pancreatic, cervical, and colorectal cancer.

High Ly6H expression and survival outcome in multiple cancers

Table 6 also showed that a high Ly6H mRNA expression in breast cancer was significantly correlated

with decreased five-year recurrence survival (recurrence, n=47 vs recurrence free, n=105) and decreased five-year metastasis free survival (metastasis, n=29 vs metastasis free, n=123) in Desmedt [128] study. High Ly6H mRNA expression in colorectal cancer was significantly correlated with decreased five-year recurrence survival (recurrence, n=56 vs recurrence free, n=30) in Jorissen [65] study.

High Ly6H mRNA expression in colorectal cancer was significantly correlated with poor five-year relapse free survival (low Ly6H, n=70; high Ly6E, n=70; HR=7.6, p=0.0326, n= number of patient, HR=hazard ratio) shown by PROGgeneV2 (Table S3, Figure 3A).

High Ly6H mRNA expression in lung cancer was significantly correlated with poor five-year first progression free survival (low Ly6H, n=557; high Ly6E, n=425; HR=1.77, p=2.90E-09), five-year post progression free survival (low Ly6H, n=87; high Ly6E, n=257; HR=1.47, p=0.015) and five-year overall survival (low Ly6H, n=484; high Ly6E, n=1442; HR=1.3, p=6.00E-04) shown by KM plotter (Table S3, Figure 3B).

High Ly6H mRNA expression in ovarian cancer was significantly correlated with poor five-year post progression free survival (low Ly6H, n=305; high Ly6E, n=506; HR=1.3, p=6.00E-04) shown by KM plotter and five-year overall survival (low Ly6H, n=96; high Ly6E, n=97; HR=1.34, p=0.034) shown by PROGgeneV2 (Table S3, Figure 3C).

High Ly6H mRNA expression in gastric cancer was significantly correlated with poor five-year first progression free survival (low Ly6H, n=336; high Ly6E, n=499; HR=1.5, p=6.9E-05) and five-year overall survival (low Ly6H, n=377; high Ly6E, n=499; HR=1.56, p=6E-07) shown by KM plotter (Table S3, Figure 3D).

These data show that high Ly6H expression was significantly correlated with poor clinical outcome in in breast, colon, lung, ovarian and gastric cancer.

Increased expression of Ly6K in multiple cancers

We investigated whether Ly6K was differentially expressed in clinical samples of cancer in multiple studies.
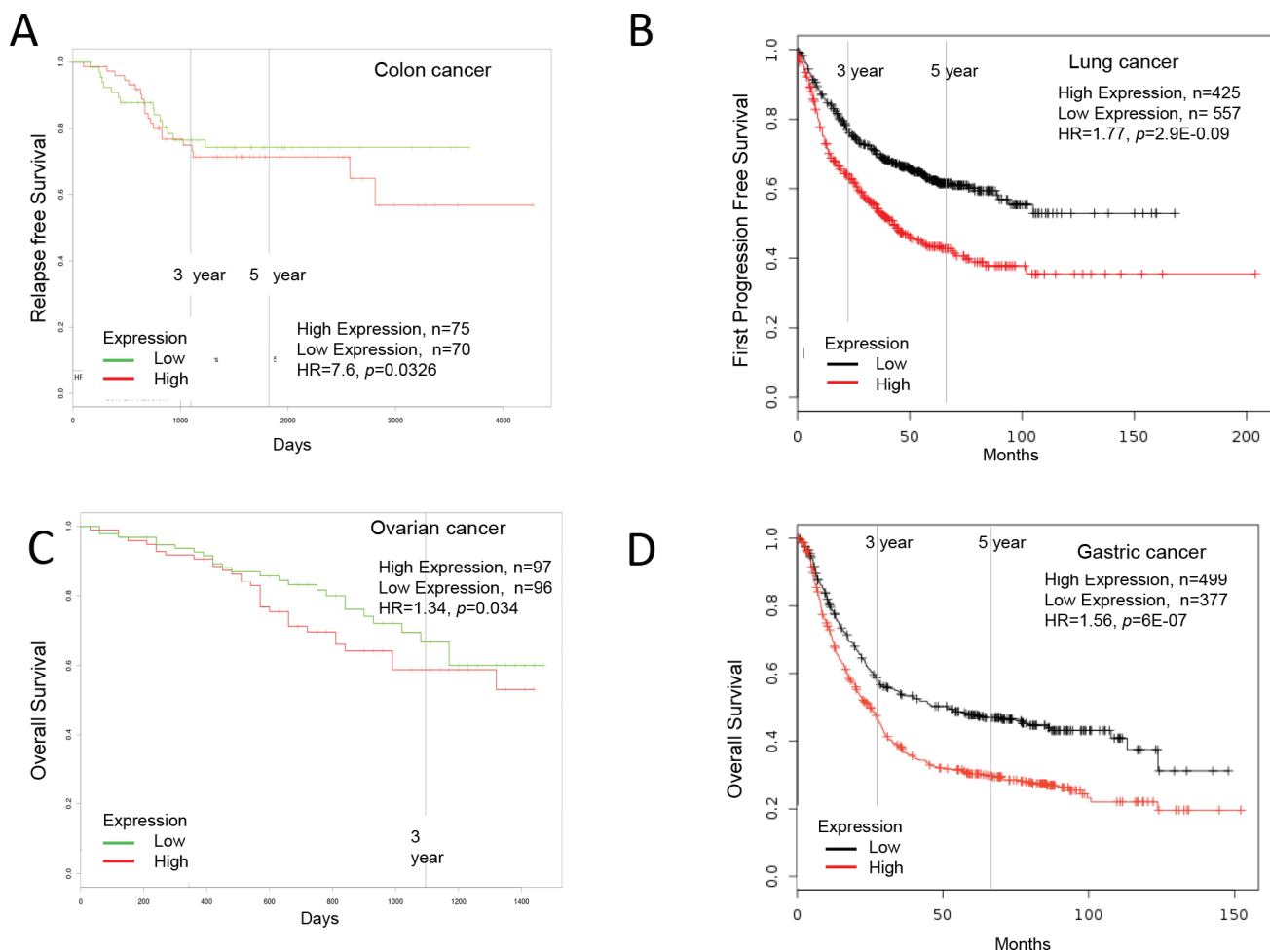

Figure 3: Increased Ly6H expression in cancer and patient survival. High Ly6H expression leads to poor survival in **A.** colon cancer, **B.** lung cancer, **C.** ovarian cancer and **D.** gastric cancer.

The data was visualized using Oncomine. As shown in Table 7, we found that Ly6K mRNA expression was significantly increased in 188 samples of bladder cancer than 68 samples of normal relevant tissue in Lee [45] studies. Ly6K mRNA expression was significantly increased in 497 samples of breast cancer than 205 samples of normal tissue in TCGA (Unpublished, NCI) and Curtis [60] study. Ly6K mRNA expression was significantly increased in 40 samples of cervical cancer than 5 samples of normal tissue in Biewenga [83] study. Ly6K mRNA expression was significantly increased in 51 samples of esophageal cancer than 51 samples of normal tissue in Su [78] study. Ly6K mRNA expression was significantly increased in 92 samples of head and neck cancer than 43 samples of normal tissue in Peng [92], He [23] and Ye [91] studies. Ly6K mRNA expression was significantly increased in 375 samples of lung cancer than 143 samples of normal tissue in Hou [31], Selamat [27], Okayama [29] studies. Ly6K mRNA expression was significantly increased in 273 samples of colorectal cancer than 71 samples of normal tissue in Sabates-Bellver [38], TCGA [132] and Skrzypczak [41] studies.

These results show that Ly6K expression was significantly increased in bladder, breast, cervical, esophageal, head and neck, lung and colorectal cancer than their counter part normal tissue.

As shown in Table 8, we found that Ly6K expression was increased in subtypes of multiple cancers. Ly6K mRNA expression was significantly higher in Myc amplified brain and CNS cancer (n=12) than cancer without Myc amplification (n=5) in Robinson [108] study. Ly6K mRNA expression was significantly higher in astrocytoma, grade 4 (n=76) than astrocytoma grade 3 (n=24) in Phillips [69] study. Ly6K expression was significantly higher in glioblastoma (n=59) than astrocytoma (n=8) in Freije [133] study. Ly6K expression was significantly higher in recurred brain tumors (n=7) than primary brain tumors (n=20) in Liang [134] study. Higher Ly6K expression was correlated with breast cancer stage as seen by the significant higher expression of Ly6K in ductal stage N1+ (n=19) than ductal stage N0 (n=20) and invasive stage N1+ (n=9) than invasive stage N0 n=22) in Julka [110], and Stickeler [47] studies. Ly6K mRNA expression was significantly higher in triple negative breast cancer (TNBC) (n=163) than non-TNBC breast cancer (n=584) in Bittner (Unpublished, GSE2109), Korde [54], TCGA (Unpublished, NCI), Julka [110], Zhao [135], Richardson2 [55] and Miyake [136] studies. Ly6K mRNA expression was significantly higher in gastric cancer grade 3 (n=18) than grade 2 (n=6) in Forster [118] study. Ly6K mRNA expression was significantly higher in TP53 mutated lung cancer (n=18) than TP53 wildtye cancer (n=23), grade 3 adenocarcinoma (n=14) than grade 2 adenocarcinoma (n=18) in Ding [137] study. Ly6K mRNA expression was significantly higher in cohort of squamous lung carcinoma (n=309) than cohorts of non small cell lung carcinoma (n=218) in TCGA [138], Bild [51], Lee [139], and Hou [31] studies. High Ly6K expression was significantly correlated with higher cancer staging in squamous lung cancer stage N1+ (n=12) than stage N0 (n=25) and in colorectal adenocarcinoma (n=15) than N0 stage in Bittner(Unpublished, GSE2109), study. Ly6K mRNA expression was significantly higher in cohort of ovarian cancer (n=8) than cohorts of precursors (n=24) in Buchholz [140] study and ovarian cancer (n=361) than borderline tumor (n=57) in Bittner(Unpublished, GSE2109), Anglesio [141] and Tothill [142] studies.

These results show that Ly6K expression was significantly increased in subtypes of brain and CNS, breast, gastric, lung, colorectal, pancreatic, and ovarian cancer.

High Ly6K expression and survival outcome in multiple cancers

Table 8 also showed a high Ly6K mRNA expression in bladder cancer was significantly correlated with decreased five-year overall survival (dead, n=33 vs alive, n=11) in Lee [45] study. High Ly6K mRNA expression in brain and CNS cancer was significantly correlated with decreased three-year overall survival (dead, n=33 vs alive, n=11) in Freije [133] study. High Ly6K mRNA expression in kidney cancer was significantly correlated with decreased one-year overall survival (dead, n=16 vs alive, n=55) and five-year overall survival (dead, n=12 vs alive, n=9) in Zhao [143] and TCGA [67] studies respectively. High Ly6K mRNA expression in breast cancer was significantly correlated with decreased three-year overall survival (dead, n=31 vs alive, n=295) and one-year overall survival (dead, n=3 vs alive n=156) in Kao [52] and Pawitan [115] studies.

High Ly6K mRNA expression in breast cancer was significantly correlated with poor five-year overall survival (low Ly6K, n=51; high Ly6K, n=52; HR=1.25, p=0.021, HR=hazard ratio, n=number of patients) shown by PROGgeneV2 (Table S4, Figure 4A). High Ly6K mRNA expression in lung cancer was significantly correlated with poor five-year relapse free survival with restriction of stage IIb cancer (low Ly6K, n=18; high Ly6K, n=21; HR=2.02, p=0.002); five year overall survival with restriction of stage IIIb cancer (low Ly6K, n=17; high Ly6K, n=18; HR=1.57, p=0.013) and with restriction of stage IIb cancer (low Ly6K, n=18; high Ly6K, n=21; HR=1.98, p=0.002) shown by PROGgeneV2 (Table S4, Figure 4B). High Ly6K mRNA expression in ovarian cancer was significantly correlated with poor five-year overall survival (low Ly6K, n=96; high Ly6K, n=97; HR=1.3, p=0.0008) shown by PROGgeneV2 (Table S4, Figure 4C). High Ly6K mRNA expression in colorectal cancer was highly correlated but not significantly associated with poor five-year relapse free survival (low

Table 7: Ly6K mRNA expression in normal and tumor tissue of multiple cancer types

Type of cancer	Reference	N (Normal)	N (Cancer)	Fold change	P-value
Bladder	[45]	68	62 (Infiltrating)	1.30	8.0E-07
			126 (Superficial)	1.20	5.1E-08
Breast	TCGA	61	76 (Invasive)	1.20	2.4E-08
			389 (Invasive Ductal)	1.32	3.2E-08
	[60]	144	32 (medullary)	1.08	3.0E-02
Cervical	[83]	5	40 (Squamous)	4.97	3.2E-11
Esphageal	[78]	51	51 (Squamous)	1.65	1.8E-06
Head and neck	[92]	22 (Oral cavity)	57 (Squamous)	1.79	1.4E-08
	[23]	9 (Thyroid)	9 (Papillary)	1.15	3.0E-03
	[91]	12 (Tongue)	26 (Squamous)	1.16	6.0E-03
Lung	[31]	65	27 (Squamous)	6.06	6.8E-12
			19 (Large cell)	1.09	1.2E-04
			45 (Adeno)	3.18	4.0E-07
	[27]	58	58 (Adeno)	1.04	7.0E-03
	[29]	20	226 (Adeno)	1.38	8.7E-04
Colorectal	[38]	7	7 (Rectal adeno)	1.82	3.0E-03
	TCGA [132]	19	29 (Colon Muc adeno)	1.21	9.0E-03
		3	24 (Cecum adeno)	1.27	2.0E-03
		3	6 (Rectal Muc adeno)	1.38	2.0E-03
		3	60 (Rectal adeno)	1.34	9.2E-04
		19	102 (Colon adeno)	1.29	9.1E-05
	[41]	24	45 (Colon adeno)	1.21	5.0E-13

Ly6K is significantly increased in bladder, breast, cervical, esophageal, head and neck, lung and colorectal cancer than their normal counterparts. Data observed using Oncomine (Invitrogen). N=number of patient samples.

Ly6K, n=60; high Ly6K, n=61; HR=13.81, p=0.059) shown by PROGgeneV2 (Table S4, Figure 4D).

These data show that high Ly6K expression was significantly correlated with poor clinical outcome in bladder, brain and CNS, kidney, breast, lung and ovarian cancer.

DISCUSSION

In this study we show that Ly6 family members (Ly6D, Ly6E, Ly6H, Ly6K) are up regulated in cancerous tissue than normal tissue, the Increased expression of these genes are heterogeneous among difference subtypes of multiple cancer and that the high expression of these genes is significantly associated with poor outcome. Recently Butte et al described that the gene expression data of tumor mass can be influenced by infiltration by immune cells and non-cancerous normal cells [144]. These contaminations may affect the analysis for gene signature associated with tumor, specifically for cells which are comprise a very little percentage of total tumor mass such as tumor infiltrating immune cells. In this study we focused on comparison of tumor vs normal. So the normal cell contamination in tumor may downplay the increased expression of Ly6 genes in tumor tissue than normal. However we observed a consistent increased expression of Ly6 in multiple studies of pan cancer. The mRNA expression data across multiple studies shows that ovarian, colorectal, gastric, breast, lung, brain and CNS, cervical, esophageal, head and neck and pancreatic cancers express significant high levels of Ly6D, Ly6E, Ly6H, Ly6K. As summarized in Table 9, the gene expression analysis showed that bladder cancer expresses significant high levels of Ly6D, Ly6E, Ly6K. The survival data for colorectal, ovarian and gastric cancer showed that all four studied genes

Table 8: Ly6K mRNA is significantly increased in subset of multiple cancer subtypes

Type of cancer	Reference	Cancer		Fold change	P-value
		N (Group 1)	N (Group 2)		
Bladder	[45]	11 (Alive at 5 years)	33 (Dead at 5 years)	1.20	3.9E-02
Brain and CNS	[108]	5 (No Myc amplification)	12 (Myc amplification)	1.26	2.5E-02
	[69]	24 (Astrocytoma Grade 3)	76 (Astrocytoma Grade 4)	1.38	2.2E-02
	[134]	20 (Primary)	7 (Recurred)	1.35	1.4E-02
	[133]	8 (Astrocytoma)	59 Glioblastoma	1.22	5.0E-03
		3 (Glioma alive at 3 year)	6 (Dead at 3 year)	1.49	9.0E-03
Kidney	[143]	55 (Alive at 1 year)	16 (Dead at 1 year)	2.11	1.2E-02
	TCGA [67]	9 (Alive at 5 years)	12 (Dead at 5 years)	1.77	3.1E-02
Breast	[110]	20 (Ductal stage N0)	19 (Ductal stageN1+)	1.64	3.0E-03
	[47]	22 (Invasive stage N0)	9 (Invasive stage N1+)	4.02	6.0E-03
	GSE2109	129 (Non-TNBC)	39 (TNBC)	2.97	1.1E-05
	[54]	39 (Non-TNBC)	21 (TNBC)	2.45	1.0E-03
	TCGA	250 (Non-TNBC)	46 (TNBC)	2.82	9.2E-05
	[52]	30 (Non-TNBC)	8 (TNBC)	2.01	1.0E-02
	[135]	28 (Non-TNBC)	5 (TNBC)	2.38	3.7E-02
	[55]	19 (Non-TNBC)	18 (TNBC)	3.74	1.0E-02
	[136]	89 (Non-TNBC)	26 (TNBC)	2.32	1.7E-02
	[135]	295 (Alive at 3 year)	31 (Dead at 3 year)	1.47	2.3E-02
	[115]	156 (Alive at 1 year)	3 (Dead at 1 year)	1.63	3.3E-02
Gastric	[118]	6 (Grade 2)	18 (Grade 3)	4.52	1.4E-02
Lung	[137]	18 (Adeno, grade 2)	14 (Adeno, grade 3)	5.48	1.2E-04
		23 (TP53 wildtype)	18 (TP53 mutation)	2.23	3.3E-02
	TCGA [138]	33 (Non-small cell)	154 (Squamous)	2.74	2.0E-03
	[51]	58 (Non-small cell)	53 (Squamous)	2.23	2.0E-03
	[139]	63 (Non-small cell)	75 (Squamous cell)	2.17	6.0E-03
	[31]	64 (Non-small cell)	27 (Squamous cell)	2.39	1.5E-04
	GSE2109	25 (Squamous N0)	12 (Squamous N1+)	2.02	1.9E-02
Colorectal	GSE2109	17 (Colon adeno N0)	15 (Colon adeno N1+)	2.00	4.5E-02
Pancreas	[140]	24 (Precursor)	8 (Cancer)	1.68	2.0E-03
Ovarian	GSE2109	10 (Borderline tumor)	146 (Cancer)	1.64	5.2E-04
	[141]	30 (Borderline tumor)	44 (Cancer)	1.54	5.0E-03
	[142]	17 (Borderline tumor)	171 (Cancer)	1.70	4.1E-06
		5 (5-year recurrence free)	103 (Recurrence at 5-year)	1.60	4.1E-02

Ly6K mRNA expression was significantly increased in subtypes of bladder, brain and CNS, kidney, breast, gastric, lung, colorectal, pancreatic and ovarian cancer. High Ly6K expression was significantly correlated with poor clinical outcome in bladder, brain and CNS, Kidney, breast, and ovarian cancer. Data observed using Oncomine (Invitrogen). N=number of patient samples.

Ly6D, Ly6E, Ly6H, Ly6K are poor prognosis markers for multiple cancer types. Survival data for bladder and brain and CNS cancer showed that Ly6E and Ly6K is poor prognosis marker for these cancers. Survival data for breast and lung cancer show that Ly6D, Ly6E and Ly6K were poor prognosis marker for these cancers. Survival data for cervical, esophageal, head and neck and pancreatic cancers in public databases were either non significant or were not available.

The mouse and human Ly6 family have a conserved LU domain (Figure 5). The LU domain is described as three-fold repeated domain in urokinase-type plasminogen activated receptors (uPAR), which occurs singly in Ly6 family [145-147]. The uPAR signaling is responsible for initiating invasion and metastasis via the activation of the plasminogen activator/plasmin cascade in breast cancers and play a role in stimulating the RAS/ERK pathway to control invasion in cancer cells [148]. The LU domain has been predicted to play a role in cancer diagnosis and malfunction of immune system [149]. It is plausible that all Ly6 family of genes may have common mechanism in tumorigenesis and their role in poor prognosis. The four genes are clustered closely at Chr8q24, the predicted transcription factor binding to their respective proximal promoter within 10KB of start site may be driven by common regulatory elements. Recently experimental validation of the important role for AP-1 activation in promoting LY6K gene expression was observed, whereas the SNP242 C allele or methylation of the CpG site was associated with reduced Ly6K
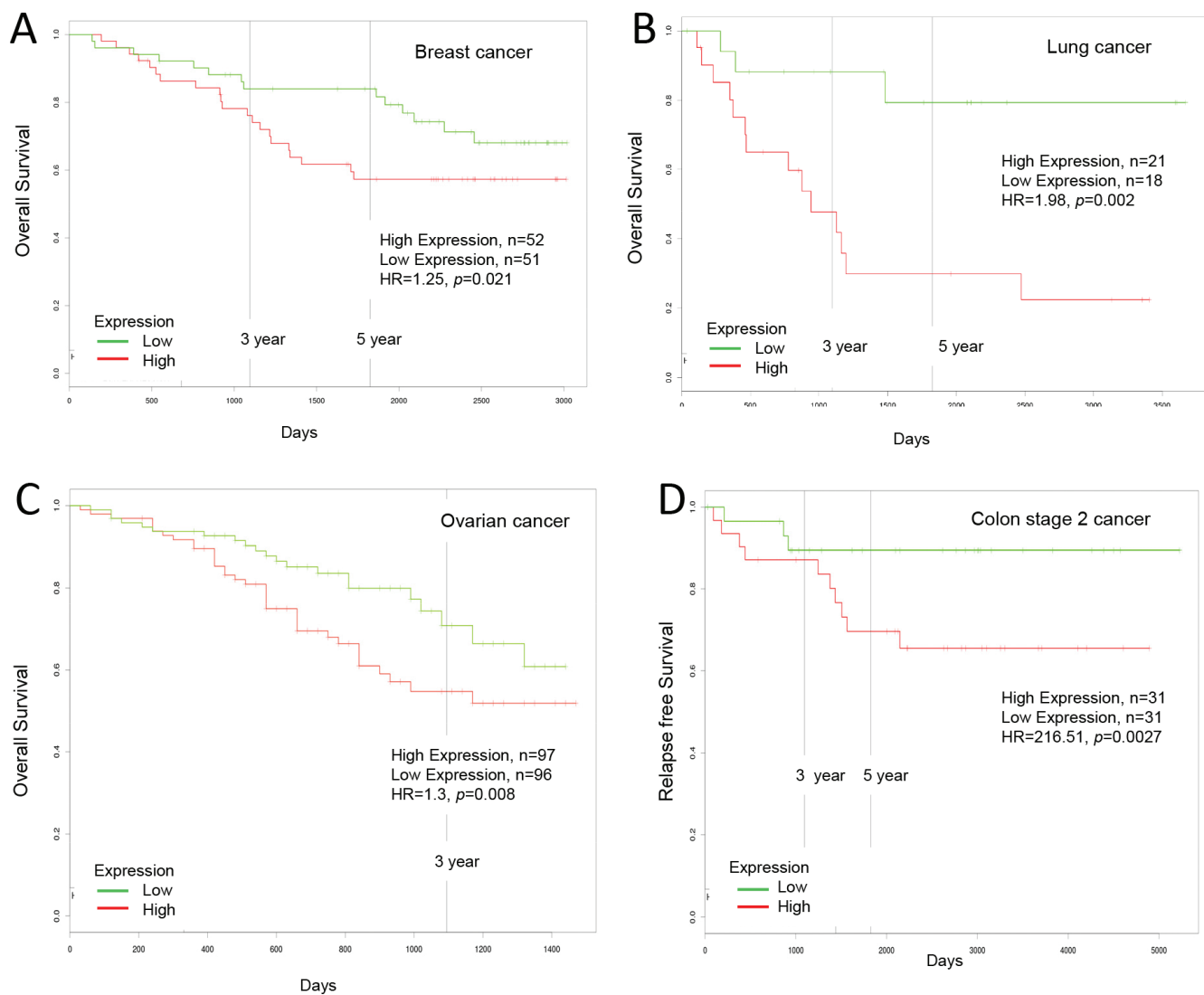

Figure 4: Increased Ly6K expression in cancer and patient survival. High Ly6H expression leads to poor survival in **A.** breast cancer, **B.** lung cancer, **C.** ovarian cancer and **D.** colon cancer.

Table 9: Correlation of high mRNA expression and patient survival outcome in multiple cancer types

Cancer Type	Genes	Expression in Tumors ($p<0.05$)	Survival Analysis ($p<0.05$)
Ovarian	LY6D	up	Poor prognosis
	LY6E	up	Poor prognosis
	LY6H	up	Poor prognosis
	LY6K	up	Poor prognosis
Colorectal	LY6D	up	Poor prognosis
	LY6E	up	Poor prognosis
	LY6H	up	Poor prognosis
	LY6K	up	Poor prognosis
Gastric	LY6D	up	Poor prognosis
	LY6E	up	Poor prognosis
	LY6H	up	Poor prognosis
	LY6K	up	Poor prognosis
Breast	LY6D	up	Poor prognosis
	LY6E	up	Poor prognosis
	LY6H	up	Poor Prognosis
	LY6K	up	Poor prognosis
Lung	LY6D	up	Poor prognosis
	LY6E	up	Poor prognosis
	LY6H	up	OS (NS), Others (NA)
	LY6K	up	Poor prognosis
Bladder	LY6D	up	OS (NS), Others (NA)
	LY6E	up	Poor prognosis
	LY6H	NS	OS (NS), Others (NA)
	LY6K	up	Poor prognosis
Brain and CNS	LY6D	up	OS (NS), Others (NA)
	LY6E	up	Poor prognosis
	LY6H	up	OS (NS), Others (NA)
	LY6K	up	Poor prognosis
Cervical	LY6D	up	OS (NA), RFS (NS)
	LY6E	up	OS (NA), RFS (NS)
	LY6H	up	OS (NA), RFS (NS)
	LY6K	up	OS (NA), RFS (NS)
Esophageal	LY6D	up	OS (NS), Others (NA)
	LY6E	up	OS (NS), Others (NA)
	LY6H	up	OS (NS), Others (NA)
	LY6K	up	OS (NS), Others (NA)
Head and neck	LY6D	up	OS (NS), Others (NA)
	LY6E	up	OS (NS), Others (NA)
	LY6H	up	OS (NS), Others (NA)
	LY6K	up	OS (NS), Others (NA)
Pancreatic	LY6D	up	OS (NS), Others (NA)
	LY6E	up	OS (NS), Others (NA)
	LY6H	up	OS (NS), Others (NA)
	LY6K	up	OS (NS), Others (NA)

OS overall survival, RFS relapse free survival, NS not significant $p<0.05$, NA no data available.
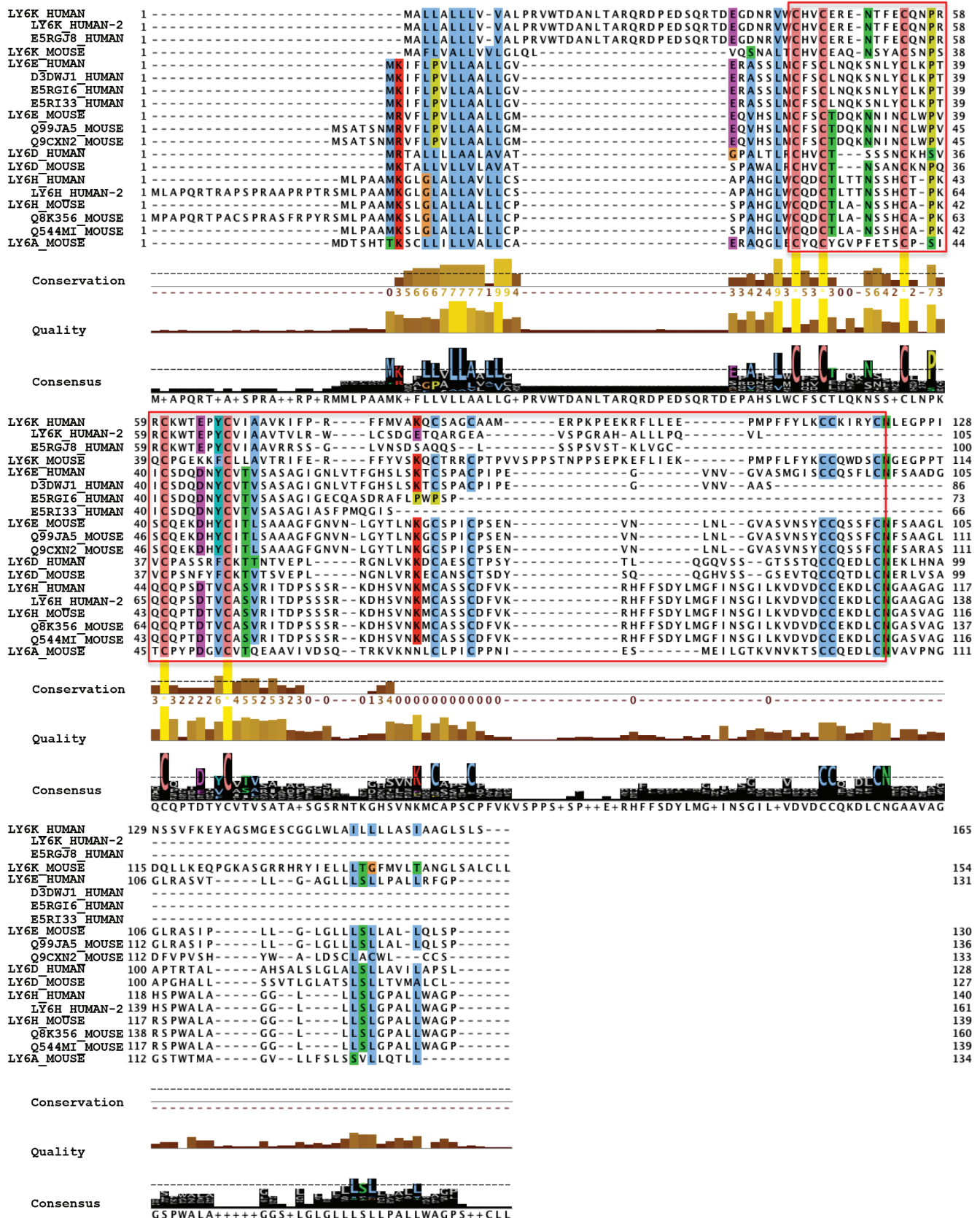
Figure 5: Ly6 gene family members have conserved LU/uPAR domain. The red box shows the region containing multiple cysteine residues that form di-sulfide bonds characteristic of the LU/uPAR domain in Ly6 family of proteins. Highlighting shows columns were the consensus sequence is present in over 50% of the aligned amino acids.

expression via inhibition of AP1 [150], suggesting additional level of complexities in regulation of Ly6 genes. Further more Ly6K, Ly6E and Ly6D expression may be regulated by multiple growth factors, nuclear receptors (Figure 6A) that can affect multitude of cellular fate including immune response, cell motility, growth, adhesion and differentiation (Figure 6B).

The Ly6K and Ly6E proteins are implicated as cancer vaccine targets and drug conjugated antibody therapy, respectively [7-9] suggesting that Ly6 family members can be used as novel candidates to develop targeted therapies. However, a detailed understanding of mechanism associated with Ly6 function is lacking. We had previously shown that Sca-1/Ly6A in mouse tumor model can inhibit TGF-β signaling by direct binding with TGF-β receptor 1 [2]. We have tested for human Ly6E and Ly6K in breast cancer and delineated the molecular mechanism behind cancer cell growth, metastasis and drug resistance (being published separately). This may explain how various members of
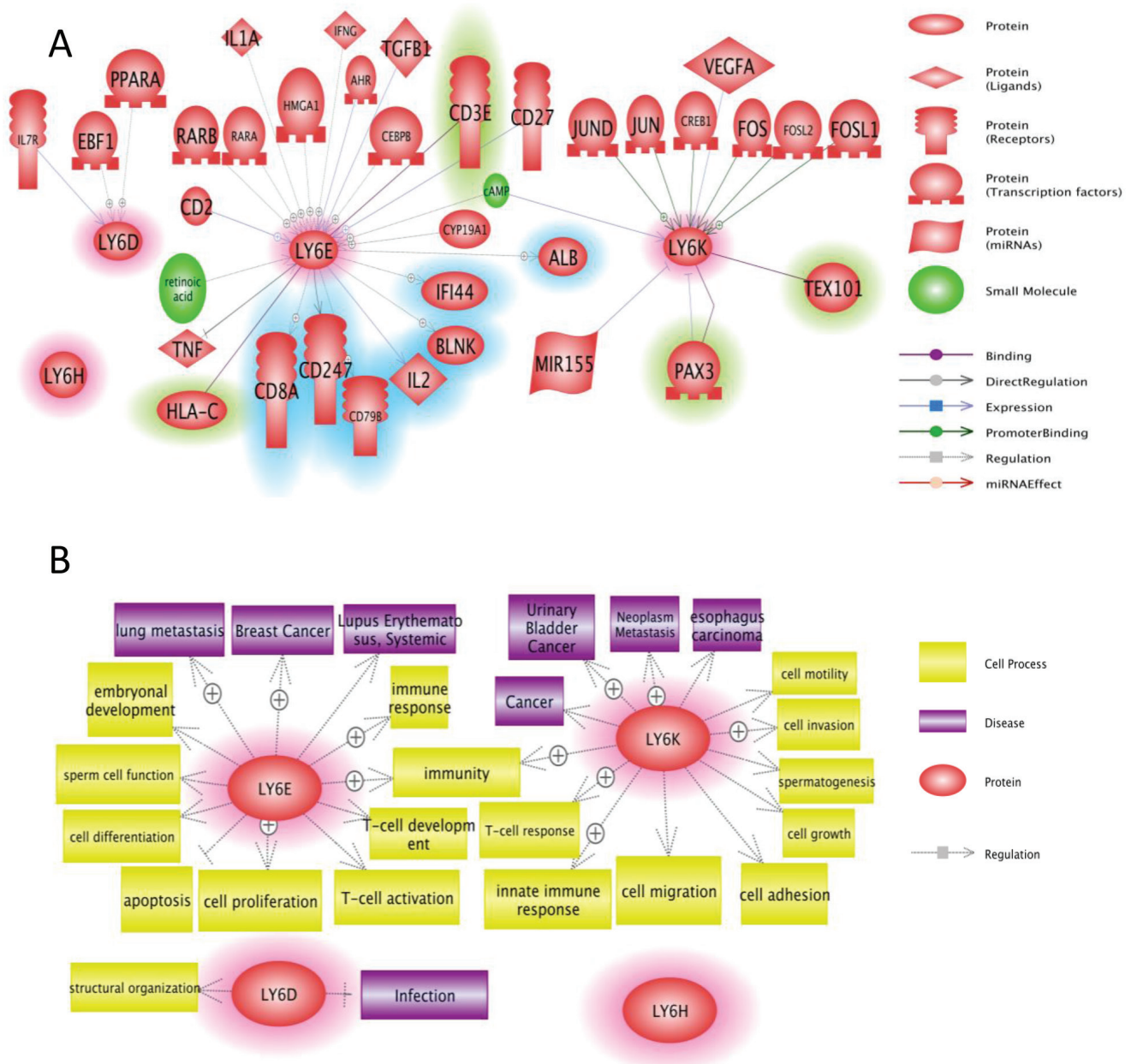
Figure 6: Network analysis of Ly6 gene family members. A. Pathway studio network analysis showed that Ly6 signaling is involved in broad range of molecules including growth factor, nuclear receptor, and micro RNAs. The upstream regulators are not highlighted, the downstream effectors are highlighted with blue, and the potential binding partners are highlighted with green. **B.** Pathway studio network analysis showed that Ly6 gene family affect multitude of cellular fate and cell-cell interaction with microenvironment ranging from growth, apoptosis, autophagy, immune response.

Ly6 gene family may be responsible for poor outcome in multiple cancers. The protein level validation for all four proteins in pan cancer is yet to be determined. We are currently in process of generating antibodies and validating commercially available antibodies using control and knockout cell lines. The protein expression of the all four genes will need to be done in pan cancer clinical samples in future studies, so that Ly6 gene family can be used as a companion diagnostic tool for multiple cancer types. The Ly6 family of genes can be a novel prognosis marker and novel candidate to develop targeted therapies.

MATERIALS AND METHODS

Bioinformatic analysis

ONCOMINE (www.oncomine.org) [151, 152] was used to visualize mRNA expression of Ly6 gene family members in different cancer types. CBioPortal (www.cbioportal.org) [153, 154] for Cancer Genomics was used to explore genetic alterations across Ly6 gene family members in different cancer types. When selecting genomic profiles, mutations and CNAs are specified by default. When available, survival analysis of LY6K and its isoforms genetic alteration in specific cancer types were selected. PathwayStudio (http://www.elsevier.com/solutions/pathway-studio) [155] was used to generate LY6K and its isoforms signaling pathway. Km plotter (kmplot.com/analysis) [156] was used to collect information about survival analysis of LY6K and its isoforms in pan-cancers. Oncomine (Invitrogen), KM plotter (http://kmplot.com/analysis/) [156] and ProgeneV2 prognostic Database (http://www.abren.net/PrognoScan/) [157] were used to collect information about survival analysis of LY6K and its isoforms in pan-cancers.

Alignment of Ly6 proteins

Protein sequences of the LY6K, LY6E, LY6D, LY6H genes from both human and mouse plus the Ly6A gene from mouse were obtained from UniProtKB [158]. In addition to the predominant protein forms, selected isoforms for each protein were also obtained. Sequences were aligned using Clustal Omega [159] with default parameters. The alignment was performed using editing and analysis into Jalview [160] platform. In the alignment presentation the small fragment proteins, redundant sequences and truncated isoforms were excluded.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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