LIQUID IMMUNOPHENOTYPING AND THE DIAGNOSIS OF LYMPHOID NEOPLASMS USING CELL-FREE RNA

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INTRODUCTION

Liquid biopsy is traditionally based on analyzing peripheral blood cell-free DNA (cfDNA). However, we recently reported that cell-free RNA (cfRNA) can also be reproducibly analyzed and quantified using next generation sequencing (NGS). The advantage of cfRNA over cfDNA are multiple. cfRNA is more sensitive than cfDNA in detecting mutations due tissue-specific expression and allele-specific expression of mutant allele. More importantly RNA expression levels reflect various biomarkers typically used in defining the phenotype of the neoplastic cells.

AIM

Using RNA expression profile as determined by targeted transcriptome we explored the potential of using cfRNA in the immunophenotyping and diagnosis of lymphoid neoplasms. We used machine learning to develop algorithms that specifically predict the immunophenotype of various lymphoid diagnostic classes and to point out the specific genes that their expression are relevant for each of the lymphoid diagnostic class.

METHOD

Peripheral blood cfDNA and cfRNA from patients with lymphoma were sequenced using 284 gene DNA panel and 1501 gene RNA panel. This included 24 samples from patients with diagnosis of mantle cell lymphoma, 22 with chronic lymphocytic leukemia (CLL), 20 with follicular lymphoma, 30 with diffuse large B-cell lymphoma, 26 other types of lymphoma. The normal control group included 51 individuals. We developed a machine learning algorithm that first selects the relative genes based on performance of each gene with cross-validation and based on stability measure using statistical significance tests. The selected genes were then used to predict the diagnostic class with k-fold cross-validation procedure (k=12).

RESULTS

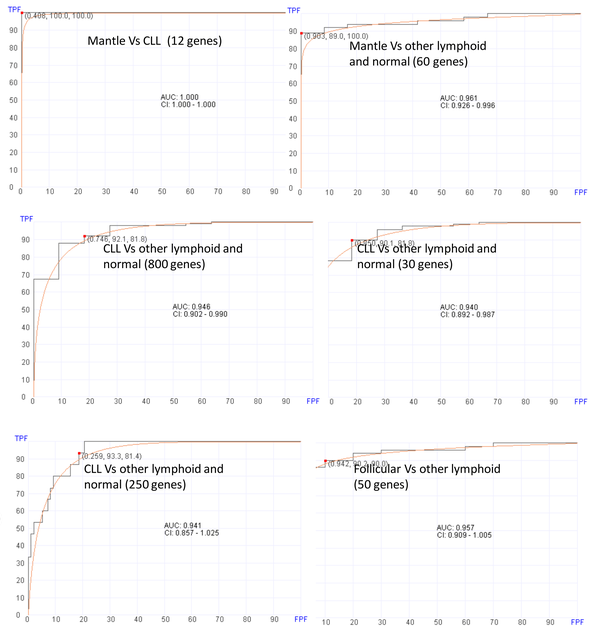
TPF, true positive fraction (sensitivity); FPF, false positive fraction (specificity).

-Significantly (P >0.0001) higher cfRNA B-cell markers (CD19, CD20, CD22, CD79A, and CD79B) than in normal control.

-There was significantly higher (P = 0.0009) CCND1 cfRNA in Mantle cell than other lymphoid neoplasms.

-CLL showed uniquely higher ratio of CD19 : CD22 cfRNA than any other lymphoid neoplasms.

-Follicular lymphoma showed significantly higher LMO2 : CD19 ratio (P=0.01) and borderline higher BCL2 : CD19 ratio (P=0.05) as compared with CLL.



cfRNA levels in the differential diagnosis of lymphoid neoplasms

Diagnostic classes	AUC	95% Confidence Interval	Sensitivity (%)	Specificity (%)	No. of genes
Mantle Vs CLL	1	1.000-1.000	100	100	12
Mantle Vs DLBCL	1	1.000-1.000	100	100	10
CLL vs Normal	1	1.000-1.000	100	100	10
CLL vs Follicular	1	1.000-1.000	100	4	5
Follicular Vs DLBCL	0.96	.870-1.000	90	93.3	30

Examples of CDs levels using cfRNA (TPM) in some hematologic neoplasms when tumor burden is high

	BCL2	CCND1	CD14	CD19	CD20	CD22	TDT	CD1A	CD2	CD3D	CD3E	CD3G	CD5	CD33	CD34	CD4	Ki67	CD10	MPO
Mantle	273.17	**4273.2**	67.09	**314.73**	**3136.3**	**2436.2**	2.95	3.86	187.77	148.27	191.75	74.06	67.33	27.21	5.17	225.23	12.58	39.81	36.58
CLL	**459.17**	1054.1	296.78	**559.51**	**69.03**	**2088.6**	30.87	201.4	287.03	183.25	114.64	113.67	**456.62**	402.68	201.78	655.99	443.31	84.74	874.35
ALL	**724.53**	1071.6	490.11	**549.63**	**54.1**	**1638**	**149.44**	114.02	209.19	142.6	136.2	119.79	350.84	439.44	**259.69**	811.42	485.11	**296.57**	701.08
DLBCL	**964.7**	855.06	359.84	**491.71**	**237.92**	**1601.9**	139.83	127.97	236.11	173.05	150.63	131.57	392.66	320.52	212.58	551.75	418.23	127.67	901.8
AML	**649.28**	803.29	**291.68**	582.29	193.69	1967.9	192.31	160.64	341.67	101.29	109	129.92	507.84	446.6	**497.32**	718.61	493.09	130.93	**1262.9**
CMML	29.89	577.69	**810.39**	0.51	0	29.93	7.42	4.06	294.94	432.9	322.69	181.57	122.94	183.23	**88.09**	415.69	47.91	749.18	29.66

CONCLUSIONS

This data demonstrates that cfRNA analysis by NGS is highly useful in liquid biopsy evaluation of myeloid neoplasms and provides information on the immunophenotype of the neoplastic process. Furthermore, when cfRNA can efficiently predict the presence of fusion genes and help in subclassification of myeloid neoplasms. Using machine learning algorithm and the cfRNA levels can provide highly accurate diagnosis and classification of myeloid neoplasms.

REFERENCES

1. Zhang H, Qureshi MA, Wahid M, Charifa A, Ehsan A, Ip A, De Dios I, Ma W, Sharma I, McCloskey J, Donato M, Siegel D, Gutierrez M, Pecora A, Goy A, Albitar M. Differential Diagnosis of Hematologic and Solid Tumors Using Targeted Transcriptome and Artificial Intelligence. Am J Pathol. 2022 Oct 13:S0002-9440(22)00313-3. doi: 10.1016/j.ajpath.2022.09.006. Online ahead of print. PMID: 36243045 Free article.

2. Ma W, Tseng R, Gorre M, Jilani I, Keating M, Kantarjian H, Cortes J, O'Brien S, Giles F, Albitar M. Plasma RNA as an alternative to cells for monitoring molecular response in patients with chronic myeloid leukemia. Haematologica. 2007 Feb;92(2):170-5. doi: 10.3324/haematol.10360. PMID: 17296565.

3. Qu KZ, Zhang K, Li H, Afdhal NH, Albitar M. Circulating microRNAs as biomarkers for hepatocellular carcinoma. J Clin Gastroenterol. 2011 Apr;45(4):355-60. doi: 10.1097/MCG.0b013e3181f18ac2.

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