

Prediction of the tumor microenvironment miRNAs targeting Natural Killer cell genes downregulated in Cancers

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ABSTRACT

Natural Killer (NK) cells are one of the important innate components of the immune system to recognize and eliminate cancer cells. However, in some instances, they become ineffective, resulting in the establishment of a cancerous state. The ineffectiveness of NK cells is due to the downregulation of genes with important functions of the immune response. The tumor microenvironments prevailing in cancerous conditions and specifically the miRNA in them may have major role in this. The present study attempts to understand how many of such miRNA, if at all, can target NK cell genes reported as downregulated in cancers. An understanding of this may provide us with the list of miRNA that would aid in predicting the immune status and further for the assessment of risk of development of a cancerous condition.

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INTRODUCTION

NK cells being the predominant innate lymphocyte cells have a major role in the surveillance of cancer cells and their subsequent elimination¹. This is brought about either by their cytotoxic activities, or by inducing apoptosis of cancer cells². Further, they also modulate adaptive immune responses for efficient cancer cell removal³. While this is a natural phenomenon, cancers sometimes establish evading this immune surveillance⁴. In several cases, evasion of immune response was associated with the down-regulation of one or a few of the genes of NK cell genes, which are known to have important functions (Table 1).

Immune evasion has been mainly attributed to the establishment of specific microenvironments that are favourable to the proliferation of cancer cells, and inhibitory to the immune cells' action against cancers⁵. miRNAs in these micro-environments have gained great importance, whose general function is to downregulate gene expression by binding to mRNA and directing them to degradation pathways. miRNA can accelerate or inhibit tumor growth, which depends on the type of gene they interact⁶. Often tumor microenvironments are known to be rich in miRNAs that enhance their growth and inhibit the immune cells to act against them. A large number of miRNAs were identified, which are overexpressed in various kinds of cancers⁷.

In the present study, we tried to probe whether the miRNAs, reported as overexpressed in cancers, target any of the genes of NK cells. For this, we have mined NK cell genes downregulated in cancers, predicted miRNA for these genes and matched the predicted miRNA with the miRNA present in cancers. Materials and methods followed for this is given below.

MATERIALS AND METHODS

- Data on NK cell genes was mined from NCBI using the key words “NK cell genes downregulated in cancer”
- miRNAs targeting NK cell genes were predicted from the “online database for prediction of functional microRNA targets” available at the -website: <https://mirdb.org/>
- Data on miRNA was mined from NCBI with the key words “miRNA and cancer”, and “miRNA in tumor micro environment”

- Using sorting tools of Excel worksheet, miRNA common in (i) tumor microenvironment and (ii) miRNA predicted for NK cell genes, were listed out.

RESULTS

Natural killer cell genes reported as downregulated in cancer condition

From NCBI search, we have identified a total of 41 genes that were reported as down-regulated in Natural killer cells under various cancer conditions” (Table 1)

Table 1			
Sl.No.	NK cell Gene	Cancer type	Reference (Pubmed ID)
1	AR	Advance prostate cancer	PMID: 23363071
2	CBL	Breast cancer	PMID: 22310290
3	CXCL12	Colon cancer	PMID: 33363463
4	CXCL8	Liver cancer,colorectal cancer	PMID: 32038715
5	ERBB2	Breast cancer	PMID: 14743203
6	Foxo1	Acute myeloid leukemia (AML), Breast cancer	PMID: 32629884
7	GJA1	Colorectal cancer	PMID: 33235496
8	HLA-B	Pancreatic cancer,stomach cancer	PMID: 11132148
9	HMGB1	Liver,lung,breast,colorectal,prostate,cervical and overian cancer	PMID: 30370641
10	ICAM1	Breast cancer	PMID: 30082828
11	IFNG	Breast cancer	PMID: 34189281
12	IGF1	Prostate cancer	PMID: 16614058
13	IL10	Breast cancer	PMID: 30324115
14	IL17a	Breast cancer	PMID: 24316750
15	IL18	Gastric cancer	PMID: 33660570
16	IL2	Metastatic renal carcinoma	PMID: 2747163
17	LEP	Breast,colorectal,prostate cancer	PMID: 27177292
18	LGALS3	Glioblastoma	

19	MAPK1	Thyroid cancer,cervical cancer,stomach cancer	PMID: 33836754
20	MAPK3	Pancreatic,breast,uterine,colorectal,bladder cancer	PMID: 33363463
21	MTOR	Breast cancer	PMID: 28400999
22	NT5E	Breast cancer	PMID: 22454080
23	PTEN	Breast cancer	PMID: 26655726
24	Ptprc	Colorectal cancer therapy	PMID: 34522210
25	RELA	Bladder cancer	PMID: 17622249
26	RUNX1	Pediatric acute lymphoblastic leukemia (ALL),AML and myelodysplastic syndrome (MDS)	PMID: 31370857
27	SMAD4	Colorectal cancer	PMID: 29483830
28	SNCA	Lung adenocarcinoma	PMID: 29300342
29	SP1	Overian cancer	PMID: 17161377
30	SPP1	Lung cancer,liver cancer	PMID: 34426769
31	Stat1	Bladder cancer	PMID: 33608980
32	STAT3	Melanoma,pancreatic cancer,cervical cancer and renal carcinoma	PMID: 30323145
33	TACR1	Colorectal cancer,uterine cancer ⁹	
34	TBX21	Lung cancer	PMID: 29615105
35	TLR4	Cervical cancer	PMID: 31287789
36	TNF	Breast, colorectal,lung cancer	PMID: 31263060
37	TP53	Breast cancer	PMID: 33133081
38	VEGFA	Metastatic breast cancer	PMID: 27110129
39	CCL2	Breast cancer,lung cancer,cervical cancer	PMID: 21187454
40	IL22	Colorectal cancer	PMID: 23379788
41	PPARG	Colorectal cancer	PMID: 33716977

Prediction of miRNAs for NK cell genes downregulated in cancers

For each of the genes in table 1, their target miRNAs were searched in the miRNA database (miRDB). This has resulted in a large number of miRNAs for every gene. These miRNAs were associated with a score. Based on miRDB guidelines, a score of 60 and above is a likely target,

and a score of 80 and above as most likely target. All the miRNAs with a score of 60 and above are listed out for further analysis. Table 2 lists the miRNA predicted for every gene with their scores. The predicted miRNAs were a total of 85, and most of them were predicted as targeting more than one gene from the list of genes in table 1.

Table 2						
NK cell	miRNAs	Score		NK cell	miRNAs	Score
AR	miR-301a-3p	90		PTEN	miR-23a-3p	99
	miR-196a-1-3p	89			miR-23b-3p	99
	miR-335-3p	73			miR-26a-5p	99
	miR-181a-5p	60			miR-29a-3p	97
	miR-181b-5p	60			miR-29b-3p	97
	miR-181d-5p	60			miR-373-5p	96
CBL	miR-200b-3p	95			miR-92a-3p	96
	miR-200c-3p	95			miR-19a-3p	91
	miR-141-3p	93			miR-19b-3p	91
	miR-200a-3p	93			miR-144-3p	88
	miR-155-5p	87			miR-335-3p	88
	miR-143-3p	69			miR-372-5p	80
	miR-96-3p	67			miR-183-5p	79
	miR-181b-2-3p	66			miR-148a-3p	76
	miR-181b-3p	66			miR-141-3p	73
	miR-221-5p	66			miR-100-3p	71
	miR-122-5p	60			miR-200a-3p	68

CCL2	miR-203a-5p	85			miR-181a-5p	67
CXCL12	miR-141-3p	95			miR-181b-5p	67
	miR-200a-3p	95			miR-181d-5p	67
	miR-144-3p	92			miR-301a-3p	65
	miR-23a-3p	90		Ptpnc	miR-335-3p	78
	miR-23b-3p	90			miR-376a-5p	73
	miR-221-3p	89			miR-205-3p	64
	miR-222-3p	89		RELA	miR-372-3p	88
	miR-335-3p	87			miR-155-5p	86
	miR-205-3p	73		RUNX1	miR-129-5p	93
	miR-19a-3p	70			miR-144-3p	91
	miR-19b-3p	70			miR-34a-3p	90
	miR-372-5p	66			miR-18a-5p	89
	miR-181b-2-3p	65			miR-18b-5p	89
	miR-181b-3p	65			miR-222-5p	85
	miR-372-3p	62			miR-141-3p	79
CXCL8	miR-376a-2-5p	80			miR-200a-3p	79
	miR-205-3p	65		miR-628-5p	77	
ERBB2	miR-193a-3p	70		miR-139-5p	67	
	miR-205-3p	67		SMAD4	miR-106a-5p	83
Foxo1	let-7a-3p	97			miR-106b-5p	83

	miR-130a-5p	87			miR-155-3p	83	
	miR-145-5p	87			miR-17-5p	83	
	let-7f-2-3p	76			miR-20a-5p	83	
	miR-144-3p	71			miR-20b-5p	83	
	let-7a-2-3p	70			miR-34a-5p	76	
	let-7g-3p	70			miR-148a-5p	73	
GJA1	miR-301b-3p	100			miR-181b-2-3p	72	
	miR-19a-3p	94			miR-125a-3p	68	
	miR-19b-3p	94			miR-345-3p	68	
	miR-205-3p	87			miR-17-3p	64	
	miR-23a-3p	87			miR-26a-5p	64	
	miR-23b-3p	87			miR-26b-5p	64	
	miR-144-3p	85			miR-19a-5p	63	
	miR-106a-3p	82			miR-19b-1-5p	63	
	miR-223-5p	78			miR-19b-2-5p	63	
	miR-125a-5p	72			miR-19a-3p	60	
	miR-125b-5p	72			miR-19b-3p	60	
	miR-122b-3p	68			SNCA	miR-106a-3p	94
	miR-148a-5p	63				miR-335-3p	83
	miR-221-5p	61				miR-223-3p	79

HLA-B	miR-10b-3p	80			miR-338-5p	78
	miR-222-5p	63			miR-216a-5p	77
HMGB1	miR-6826-3p	83			miR-345-3p	68
	miR-106a-3p	80			let-7a-2-3p	62
	miR-19a-3p	78			let-7g-3p	62
	miR-19b-3p	78			miR-126-5p	61
	miR-148a-5p	75			miR-196a-3p	61
	miR-148a-5p	75			miR-29a-3p	93
	let-7a-2-3p	73			miR-29b-3p	93
	let-7g-3p	73			miR-1290	91
ICAM1	miR-223-3p	75			let-7g-3p	86
	miR-133a-5p	70			miR-155-5p	84
IFNG	miR-24-3p	94			miR-196a-3p	84
	miR-183-5p	77			let-7a-2-3p	83
IGF1	miR-324-3p	99		SP1	miR-133a-3p	80
	miR-324-5p	99			miR-130a-5p	77
	miR-29b-1-5p	98			miR-335-5p	77
	miR-19a-5p	92			miR-100-3p	75
	miR-205-3p	92			miR-345-3p	75
	miR-29a-3p	86			miR-17-3p	69
	let-7g-5p	79			miR-24-3p	69
	miR-1290	79			miR-223-3p	61

	miR-155-3p	79	SPP1	miR-181a-5p	98
	miR-6875-3p	77		miR-181b-5p	98
	miR-148a-3p	76		miR-181d-5p	98
	miR-18a-5p	73		miR-130a-5p	93
	miR-19b-2-5p	72		miR-130b-5p	90
	miR-19b-3p	72	Stat1	miR-143-5p	78
	miR-19a-3p	68	STAT3	miR-196a-1-3p	91
	miR-335-3p	65		miR-106a-5p	87
	miR-29b-3p	63		miR-106b-5p	87
IL10	let-7a-5p	76		miR-17-5p	87
	let-7g-5p	76		miR-20a-5p	87
IL17a	miR-19a-5p	82		miR-125b-5p	75
	miR-19b-1-5p	82		miR-18a-3p	67
	miR-19b-2-5p	82		miR-17-3p	64
	miR-200a-5p	71		miR-29a-3p	63
	miR-200b-5p	71	TACR1	miR-335-3p	87
IL18	miR-197-3p	60		miR-125b-5p	67
IL2	miR-181a-5p	98	TBX21	miR-6875-3p	66
	miR-181b-5p	98		miR-141-3p	72
	miR-181d-5p	98		miR-200a-3p	72
	miR-15b-3p	66		miR-20b-3p	68

IL22	miR-29b-2-5p	75			miR-205-5p	67
	miR-203a-3p	63			miR-338-5p	88
LEP	miR-29a-3p	79		TLR4	miR-23b-5p	72
	miR-29b-3p	79			miR-23a-5p	64
	miR-200a-3p	70			miR-216b-5p	61
	miR-212-5p	65			miR-335-3p	60
	miR-345-3p	64		TNF	miR-34a-3p	96
	miR-141-3p	62			miR-34a-3p	96
	miR-29b-2-5p	61			miR-181a-5p	86
LGALS3	miR-216a-3p	75			miR-181b-5p	86
	miR-376a-5p	69			miR-181d-5p	86
MAPK1	miR-212-3p	97		TNF	miR-181a-5p	86
	miR-106b-5p	91			miR-181b-5p	86
	miR-106a-5p	90			miR-181d-5p	86
	miR-17-5p	90			miR-301a-3p	75
	miR-20a-5p	90			miR-130a-3p	75
	miR-10b-3p	89			miR-130b-3p	75
	miR-19a-3p	88			miR-181a-2-3p	72
	miR-19b-3p	88		miR-130a-5p	62	
	miR-181a-5p	79		TP53	miR-223-3p	71
	miR-181b-5p	79			let-7a-5p	60
miR-181d-5p	79		let-7g-5p		60	

	miR-183-3p	75	VEGFA	miR-15b-5p	95
	miR-92a-1-5p	75		miR-29a-3p	94
	let-7f-2-3p	72		miR-29b-2-5p	94
	miR-203a-3p	64		miR-29b-3p	94
	miR-885-5p	63		miR-200b-3p	93
	miR-196a-3p	61		miR-200c-3p	93
MAPK3	miR-92a-2-5p	82		miR-141-5p	69
	miR-212-3p	62		miR-106a-5p	66
MTOR	miR-96-5p	97		miR-106b-5p	66
	miR-100-5p	93		miR-20a-5p	66
	let-7f-2-3p	91		miR-17-5p	65
	miR-144-3p	86			
	miR-146b-3p	60			
NT5E	miR-148a-5p	89			
	miR-193a-3p	89			
PPARG	miR-130a-3p	90			
	miR-130b-3p	90			
	miR-301a-3p	90			

miRNAs reported as elevated in tumor/cancer conditions

From the literature review, we have shortlisted 77 miRNA which were reported as over expressed/elevated in various tumor micro environments or cancer conditions (Table 3).

Sl.No.	miRNA	Cancer type	Reference (Pubmed ID)
1	Let-7a	Hepatocellular cancer	PMID: 18817506
2	let-7f-2	Ovarian cancer	as per NCBI
3	Let-7g	Ovarian cancer	PMID: 30952937
4	miR-10b	Pancreatic cancer	PMID: 22018284

5	miR-15b	Gastric cancer, colorectal cancer and Esophageal squamous cell carcinoma.	PMID: 33363587
6	miR-17	Lung cancer	PMID: 30443163
7	miR-18	Hepatocellular carcinoma	PMID: 30519035
8	miR-18a	Prostate cancer	PMID: 24752237
9	miR-19a	CTC, Lung Cancer	PMID: 23824915
10	miR-19b	CTC, Lung cancer	PMID: 23824915
11	miR-20a	Gastrointestinal cancer	PMID: 32410584
12	miR-21	CTC, Breast cancer, Lung cancer, Pancreatic cancer, Prostate cancer, Hepatocellular cancer	PMID: 32244168
13	miR-22	CTC, Breast cancer stemness	PMID: 25304371
14	miR-23a	Acute myeloid leukemia (AML), bladder cancer, glioma, HCC, MPM, and pancreatic adenocarcinoma	PMID: 30577536
15	miR-23b	Renal cancer	PMID: 23189187
16	miR-24	Prostate cancer	PMID: 28157714
17	miR-26a	Breast cancer	PMID: 22848262
18	miR-29a	Lung cancer	PMID: 30788428
19	miR-29b	Renal cancer	PMID: 26823729
20	miR-32	Prostate cancer	PMID: 35228520
21	miR-34	Liver cancer, lung cancer, breast cancer, prostate cancer, colorectal cancer	PMID: 30717802
22	miR-34a	Thyroid cancer	PMID: 32764498
23	miR-92a	Colorectal, gastric, breast, lung and hepatocellular cancer, non-muscle invasive bladder cancer, esophageal squamous cell carcinoma, and epithelial ovarian cancer	PMID: 31632984
24	miR-96	Colorectal cancer	PMID: 18817506
25	miR-100	Pancreatic cancer	PMID: 18817506
26	miR-106a	pancreatic cancer	PMID: 24444603
27	miR-106b	Prostate cancer	PMID: 35685464
28	miR-122	hepatocellular carcinoma	PMID: 18817506
29	miR-125b	Pancreatic cancer, Prostate cancer	PMID: 22898264
30	miR-126	Lung cancer	PMID: 18817506
31	miR-129	Bladder cancer	PMID: 29378184
32	miR-130	CTC	PMID: 26837847
33	miR-133a	CTC	PMID: 25620172
34	miR-139-3p	Breast cancer	PMID: 31485677
35	miR-141	CTC, Ovarian cancer	PMID: 18817506
36	miR-143	Cervical cancer	PMID: 18817506
37	miR-144	Lung cancer	PMID: 25660220
38	miR-145	prostate, bladder, colon, ovarian, and esophageal cancers	PMID: 30774425
39	miR-146b	Thyroid cancer	PMID: 35186709

40	miR-148a	CTC, Prostate cancer	PMID: 34630712
41	miR-155	CTC, Pancreatic cancer	PMID: 29893326
42	mir-181a	Pancreatic cancer	PMID: 18817506
43	miR-181b	Pancreatic cancer, Thyroid cancer	PMID: 18817506
44	miR-181d	Pancreatic cancer	PMID: 18817506
45	miR-183	Colorectal cancer	PMID: 18817506
46	miR-193a-3p	Colorectal cancer	PMID: 32095323
47	miR-196a	Pancreatic cancer	PMID: 18817506
48	miR-197	Thyroid cancer	PMID: 18817506
49	miR-200	Pancreatic cancer	PMID: 24262661
50	miR-200a	Ovarian cancer	PMID: 18817506
51	miR-200b	Breast cancer	PMID: 32256756
52	miR-200c	Colon cancer	PMID: 18817506
53	miR-203	Pancreatic cancer	PMID: 33109107
54	miR-205	Lung cancer	PMID: 18817506
55	miR-212	Pancreatic cancer	PMID: 18817506
56	miR-216	Pancreatic cancer	PMID: 30393486
57	miR-221	CTC, Breast cancer stemness, Pancreatic cancer, Prostate cancer, Bladder cancer, Hepatocellular cancer, Thyroid cancer	PMID: 18817506
58	miR-222	Pancreatic cancer, Prostate cancer, Thyroid cancer	PMID: 18817506
59	miR-223	Acute myeloid leukemia (AML)	PMID: 33951281
60	miR-224	Hepatocellular cancer	PMID: 18817506
61	miR-301	Pancreatic cancer	PMID: 18817506
62	miR-324-3p	Lung cancer	PMID: 29844840
63	miR-335	breast cancer, lung cancer, colorectal cancer, and ovarian cancer	PMID: 34045870
64	miR-338-5p	Colorectal cancer	PMID: 30982765
65	miR-345	Breast cancer	PMID: 34959269
66	miR346	Thyroid cancer	PMID: 21687652
67	miR-372	Breast cancer	PMID: 29456685
68	miR-376a	Pancreatic cancer	PMID: 18817506
69	miR-409-3p	Breast cancer	PMID: 30250593
70	miR-431	Breast cancer	PMID: 31897213
71	miR-521	Prostate cancer	PMID: 18668526
72	miR-628-5p	Colorectal cancer	PMID: 32016467
73	miR-885-5p	Hepatocellular carcinoma	PMID: 33751897
74	miR-886-3p	small cell lung cancer	PMID: 30230945
75	miR-1290	Colorectal cancer	PMID: 34897783
76	miR-6826	Pancreatic-biliary cancer	PMID: 27878288
77	miR-6875	hepatocellular carcinoma	PMID: 30621734

Presence of miRNAs predicted as targets for NK cell genes, in tumor micro-environment / cancer conditions

By merging and sorting tables 2 and 3, a total of 59 miRNAs were identified which were common in both the tables, that is (i) the miRNA predicted as targets for NK cell genes downregulated in cancers and (ii) the miRNAs present in the tumor microenvironment. Among them 49 miRNA were predicted as targeting more than one gene. The miRNA which were targeting maximum number of genes were miRNA 19a (10 genes), miRNA 181b (9 genes), and miRNA 335(9 genes). There were 16 miRNA which were predicted to target 5-7 genes, and 30 miRNA, which were predicted to target 2-4 genes. Eight miRNA were predicted to target single gene (Table 4).

miRNAs common in tables 2 & 3)	No of target genes	Gene target	Predicted Score	miRNAs common in tables 2 & 3)	No of target genes	Gene target	Predicted Score
Let-7a	3			miR-197	1		
		Foxo1	97			IL18	60
		IL10	76	miR-19a	10		
		TP53	60			CXCL12	70
let-7f-2	3					GJA1	94
		Foxo1	76			HMGB1	78
		MAPK1	72			IGF1	68
		MTOR	91			MAPK1	88
Let-7g	7					PTEN	91
		Foxo1	70			SMAD4	60
		HMGB1	73			IGF1	92
		SNCA	62			IL17a	82
		SP1	86	miR-19b	7		
		IGF1	79			SMAD4	63
		IL10	76			CXCL12	70
		TP53	60			GJA1	94
miR-100	3					HMGB1	78
		PTEN	71			IGF1	72
		SP1	75			MAPK1	88
		MTOR	93			PTEN	91
miR-106a	7					SMAD4	60
		GJA1	82	miR-200a	7		

		HMGB1	80			CBL	93
		SNCA	94			CXCL12	95
		MAPK1	90			LEP	70
		SMAD4	83			PTEN	68
		STAT3	87			RUNX1	79
		VEGFA	66			TBX21	72
miR-106b	4					IL17a	71
		MAPK1	91	miR-200b	3		
		SMAD4	83			CBL	95
		STAT3	87			VEGFA	93
		VEGFA	66			IL17a	71
miR-10b	2			miR-200c	2		
		HLA-B	80			CBL	95
		MAPK1	89			VEGFA	93
miR-122	1			miR-205	7		
		CBL	60			CXCL12	73
miR-125b	3					CXCL8	65
		GJA1	72			ERBB2	67
		STAT3	75			GJA1	87
		TACR1	67			IGF1	92
miR-126	1					Ptprc	64
		SNCA	61			TBX21	67
miR-1290	2			miR-20a	4		
		IGF1	79			MAPK1	90
		SP1	91			SMAD4	83
miR-133a	2					STAT3	87
		SP1	80			VEGFA	66
		ICAM1	70	miR-212	3		
miR-139	1					MAPK1	97
		RUNX1	67			MAPK3	62
miR-141	7					LEP	65
		CBL	93	miR-221	3		
		CXCL12	95			CXCL12	89
		LEP	62			CBL	66
		PTEN	73			GJA1	61
		RUNX1	79	miR-222	3		
		TBX21	72			CXCL12	89
		VEGFA	69			HLA-B	63
miR-143	2					RUNX1	85
		CBL	69	miR-223	5		
		Stat1	78			ICAM1	75
miR-144	6					SNCA	79
		CXCL12	92			SP1	61

		Foxo1	71			TP53	71
		GJA1	85			GJA1	78
		MTOR	86	miR-23a	5	CXCL12	90
		PTEN	88			GJA1	87
		RUNX1	91			PTEN	99
miR-145	1					TLR4	64
		Foxo1	87	miR-23b	4		
miR-146b	1					CXCL12	90
		MTOR	60			GJA1	87
miR-148a	7					PTEN	99
		IGF1	76			TLR4	72
		PTEN	76	miR-24	2		
		GJA1	63			IFNG	94
		HMGB1	75			SP1	69
		HMGB1	75	miR-26a	2		
		NT5E	89			PTEN	99
		SMAD4	73			SMAD4	64
miR-155	5			miR-29a	6		
		IGF1	79			IGF1	86
		SMAD4	83			LEP	79
		CBL	87			PTEN	97
		RELA	86			SP1	93
		SP1	84			STAT3	63
miR-15b	2					VEGFA	94
		IL2	66	miR-29b	5		
		VEGFA	95			IGF1	63
miR-17	7					LEP	79
		SMAD4	64			PTEN	97
		SP1	69			SP1	93
		STAT3	64			VEGFA	94
		MAPK1	90	miR-324	2		
		SMAD4	83			IGF1	99
		STAT3	87			IGF1	99
		VEGFA	65	miR-335	9		
mir-181a	7					AR	73
		AR	60			CXCL12	87
		IL2	98			IGF1	65
		MAPK1	79			PTEN	88
		PTEN	67			Ptprc	78
		SPP1	98			SNCA	83
		TNF	86			TACR1	87
		TNF	86			TLR4	60
miR-181b	9						

		CBL	66			SP1	77
			65		miR-338-5p		
		CXCL12				SNCA	78
		AR	60			TLR4	88
		IL2	98				
		MAPK1	79		miR-345	4	
		PTEN	67			LEP	64
		SPP1	98			SMAD4	68
		TNF	86			SNCA	68
miR-181d	7	TNF	86			SP1	75
		AR	60		miR-34a	4	
		IL2	98			RUNX1	90
		MAPK1	79			TNF	96
		PTEN	67			TNF	96
		SPP1	98			SMAD4	76
		TNF	86		miR-372	4	
		TNF	86			CXCL12	62
miR-183	3					RELA	88
		MAPK1	75			CXCL12	66
		IFNG	77			PTEN	80
		PTEN	79		miR-376a	2	
miR-18a	3					LGALS3	69
		STAT3	67			Ptprc	73
		IGF1	73		miR-628	1	
		RUNX1	89			RUNX1	77
miR-193a	2				miR-6826	1	
		ERBB2	70			HMGB1	83
		NT5E	89		3p	2	
miR-196a	3					IGF1	77
		MAPK1	61			TACR1	66
		SNCA	61		miR-885-5p		
		SP1	84			MAPK1	63
					miR-96	2	
						CBL	67
						MTOR	97

Among the 41 NK cell genes, which were reported as downregulated, 39 of them were predicted to have miRNA targets that are seen in various cancer types. Several of the above genes were seen to be targeted by multiple miRNA. About 9 genes were targeted by 10-19 miRNAs, 8 genes were

targeted by 5-8miRNAs, and 16 genes were targeted by 2-4 miRNAs. 5 genes were targeted by single miRNA (Table 5).

Table 5			
Gene	Number of miRNAs targeting the gene	Gene	Number of miRNAs targeting the gene
PTEN	18	IL17a	3
IGF1	15	Ptprc	3
MAPK1	15	SPP1	3
CXCL12	14	TACR1	3
SMAD4	13	TBX21	3
SP1	13	TP53	3
GJA1	11	ERBB2	2
CBL	10	HLA-B	2
VEGFA	10	ICAM1	2
RUNX1	8	IFNG	2
SNCA	8	IL10	2
STAT3	8	NT5E	2
TNF	8	RELA	2
HMGB1	7	CXCL8	1
LEP	6	IL18	1
Foxo1	5	LGALS3	1
MTOR	5	MAPK3	1
AR	4	Stat1	1
IL2	4		
TLR4	4		

DISCUSSION

As evidenced from the results, there are several genes reported as down regulated in NK cells in cancer conditions. The specific microenvironments, which might have established over a period and specifically the miRNA in such microenvironment, could have a major role in their down regulation. In the present study, we have predicted miRNA for the down-regulated genes of NK cells and found that the majority of these predicted miRNA were reported as elevated in different types of cancers. It is interesting to note that several of the above miRNA, targeted multiple genes. Further it is evidenced that some of the downregulated genes in NK cells can be targeted by multiple miRNAs, which were reported as elevated in cancer conditions. The genes down-regulated in NK cells, are known to have important roles in development, maturation, and launch of immune response (Table 6)

Table 6

Gene	Role	Reference No.
PTEN	: NK cells deficient in PTEN, were found to be defective in their migration towards the tumor site.	8
IGF1	: increase cytotoxic effector function	9
MAPK1	: Deficiency of MAPK1 was shown to suppress NK cell cytotoxic activity.	10
CXCL12	: The downregulation of CXCL12 was found to suppress immune response and increase metastasis	11
SMAD4	: cell homeostasis and maturation, to prevent tumor spread and preserve anti-tumor immunity.	12
SP1	: From KEGG, SP1 was found to network, RIG-I-IRF7/3 signaling pathway, IFN signaling pathway	Kegg network
GJA1	: Is known to mediate intercellular communication to NK cell activation and modifies NK cytotoxic capacity.	13
CBL	: The expression of Cbl-b is significantly upregulated in primary human NK cells activated by IL-15, IL-2, and the human NK cell-sensitive tumor cell line K562 that lacks MHC class I expression. Downregulation of Cbl-b resulted in significant increases in granzyme B and perforin expression, IFN- γ production, and cytotoxic activity against tumor cells.	14
VEGFA	: it may contribute to vascular stability and maturation.	15
RUNX1	: Implicated in the differentiation of NK cells.	16
SNCA	: From KEGG, SNCA networks (i) 26S proteasome-mediated protein degradation, (ii) VGCC-Ca ²⁺ -apoptotic pathway, (iii) ATF6-mediated transcription, (iv) IRE1a-XBP1 signaling pathway, (v) PERK-ATF4 signaling pathway, (vi) L-DOPA generation, (vii) transport of dopamine, (viii) electron transfer in Complex I, (ix) anterograde axonal transport	Kegg network
STAT3	: STAT3 aids tumor cells to escape natural killer (NK) cell-mediated immune surveillance. NK cells are innate lymphocytes, which can directly kill malignant cells but also regulate adaptive immune responses and contribute to the composition of the tumor microenvironment.	17
TNF	: gets rid of cancer cells by producing significant inflammatory cytokines	

HMGB1	:	important cytotoxic compound of the innate immune system, affecting the energy metabolism of diverse pathogens and particularly of cancer cells through inhibition of tetrameric	18
			19
Foxo1	:	implicated in the development and function of lymphocytes	20
MTOR	:	mTOR-deficient NK cells were hyporesponsive to bacterial and viral stimulation	21
AR	:	targeting AR could enhance natural killer (NK) cell tumor-killing efficacy by decreasing PD-L1 expression.	22
TLR4	:	Recognize DAMP signals. Though TLR ligands alone are unable activate NK cells	23
IL17a	:	IL17 acts as the checkpoint during NK cell terminal maturation	24
Ptprc	:	negative regulator of both NK cell homeostasis	25
SPP1	:	SPP1 can regulate the host immune system via upregulating IL-12 and IFN γ in mouse macrophages	26
TBX21	:	development and differentiation	27
TP53	:	recognition and elimination of cancerous cells	28
ERBB2	:	human NK cells expressing an ErbB2-specific chimeric antigen receptor (CAR)	29
HLA-B	:	NK cell mediated lysis	30
ICAM1	:	NK cell infiltration contrasts with the high concentrations of adhesion molecules (ICAM-1 and VCAM-1) and high local levels of NK cell-attracting chemokines detected within the malignant tissue. Thus, unknown mechanisms might exclude NK cells from solid tumors.	31
IFNG	:	stimulation through the low-affinity Fc-binding receptor CD16	32
IL10	:	increases the expression of activation and cytotoxicity-related genes in NK cells	33
NT5E	:	overexpressed in glioblastoma (GBM), where it contributes to the tumor's pathophysiology via the generation of immunosuppressive adenosine	34
RELA	:	Normal NKT cell cytokine production.	35
CXCL8	:	modulate immune responses	36
IL18	:	Adaptive NK cells show an impaired responsiveness to cytokines that are involved in the early phases of an anti-viral response	37
LGALS3	:	The functional roles of galectins in cellular response to membrane damage are rapidly expanding	38
Stat1	:	act downstream of other cytokine receptors like the type I IFN receptor	39

As can be seen from the above, most of the genes have important functions in NK cells, and the downregulation of these can severely hamper the immune response.

Lately, many strides have been made for an effective immune response against cancers by introducing novel immuno-therapies, most of which target a single type of cancer cell antigen. However, it is also equally important to restore or even enhance natural immunity, by various means, ignorance of which, even if brings temporary results from above, can stand a risk of relapse.

In our present study, we made an effort to identify a set of markers, which may aid in understanding the effectiveness of an individual's natural response to cancer, and hence in predicting the risk of development of a cancerous condition. This set of markers may prove to be a valuable tool upon their assessment through clinical studies.

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