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Evaluating the Basis for NK Sensitivity and NK Resistance in Prototypic NK Sensitive and Resistant Cell Line

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Keywords

- P815 cell
- Natural killer cells receptors
- YAC-1 cells
- Ligand interactions

Abstract

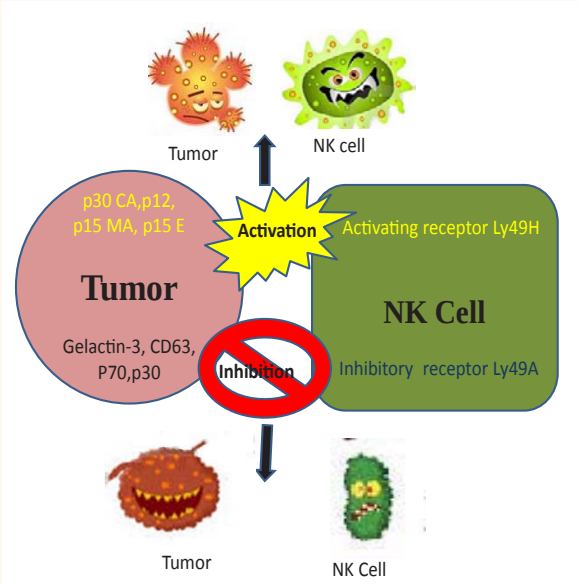
Tumors develop numerous mechanisms through which they evade NK cell attack. Several molecules secreted by YAC-1 cells and P815 cells were evaluated for their interaction with NK cell receptors. The affinity characterization of NK cell receptor with tumor expressed ligands will help in understanding the binding patterns required for the activation of NK cell activity. A detailed understanding of the mechanisms of tumor evasion and tumor susceptibility in the tumor microenvironment is essential for developing effective cancer therapies. The multi-faceted recognition pattern of tumor derived factors by NK activating receptors and every accessible surface involved in this binding event has been explored. Interaction between the known factors (galactin-3, CD63 (AD1 antigen), gp70 and p30) present on the surface membrane of mastocytoma P815 cell with NK inhibitory receptor Ly49A on NK cell was explored for evaluating the basis for tumor resistance to NK mediated lysis and interaction between T-cell lymphoma YAC-1 cell derived ligands(p30CA, p12, p15MA and p15E) with NK activating receptor Ly49H was evaluated for potential basis for NK susceptibility by YAC cells addressed by computational approaches which further help to develop NK based cancer therapeutic strategies (Graphic 1).

INTRODUCTION

NK cells are large, granular, cytotoxic lymphocytes of innate immune system. NK cells have natural capacity to kill tumor cell. They do not require any prior antigenic sensitization and is capable of dealing with a broad range of virus infected cells and tumor cells. NK cell activation and function are strongly influenced by the interplay of inhibitory and activating signals [1].

Tumor cells have evolved various mechanisms by which they can evade NK cell attack. These mechanisms include interference of NK cell activation, inducing inhibition as well as modulation of NK receptor expression pattern and thus regulating NK cell function. Tumor derived factors may cause modulation of co-stimulation, adhesion or susceptibility to apoptosis [2].

NK cells recognize target cells in two different ways i.e. lack of MHC-class I expression and absence of expression of appropriate ligands for NK cell receptor which renders target cells susceptible to NK mediated lysis. NK cell receptors utilize several parallel recognition patterns that enable them to distinguish between abnormal cell and healthy cell.



Graphic 1 Tumor and NK cell interaction.

YAC-1 is a murine T-cell lymphoma cell line which is susceptible to NK-mediated killing. MHC-I molecule is a ligand for inhibitory receptors on NK cells [3], thus causing susceptibility of YAC-1 cells to NK cell cytotoxicity, while P815, which is a mastocytoma cell line expresses reduced activating ligands. Inhibitory receptor present on NK cells MHC molecule and upon engagement of these receptors, block the ability of NK cells to attack target cells, hence if inhibitory ligand is not present on the target cell, NK cell will cause lysis of the target cell.

However in the present study we tried to explore if in the addition to modulation of MHC I (ligand for inhibitory receptor) direct binding to tumor expressed ligand with other NK inhibitory or activating receptors do indeed play a role in NK resistance and susceptibility.

Structure of various ligands expressed on YAC-1 and P815 cell were modeled using Phyre 2 and molecular docking was performed using PATCHDOCK between YAC-1 ligands (namely, p30CA,p12,p15MA and p15E) and NK activating receptor, Ly49H,similarly P815 ligands (namely, galectin-3, CD63, gp70 and p30) were studied for molecular docking with NK inhibitory receptor Ly49A.

In the present study we tried to elucidate the molecular basis of susceptibility of YAC tumor cells and resistance of P815 cells to NK mediated cytotoxicity. YAC 1 cells express low level of MHC I (ligand for inhibitory receptors on NK) and P815 cells on the other hand express high level of MHC I molecules thus causing susceptibility of YAC and resistance of P815 to NK mediated cytolysis. However, in the present study in addition to above expression pattern of MHC I, we show direct binding efficiency of tumor expressed ligands with activating and inhibitory NK receptors. Furthermore, D-complex energy was calculated and some significant interaction between ligand and receptor, which highlighted the binding pattern required for the activation of NK cell effecter function has been reported in present study.

MATERIAL AND METHODS

Receptor Modeling

Ly49H: A 265 amino acid sequence of Ly49H of mouse origin was retrieved from NCBI (accession no. = AAR03586.1) and Ly49A: A 262 amino acid sequence of Ly49A of mouse origin was retrieved from NCBI (accession no = AAF99547.1). Since the crystal structure of Ly49H and Ly49A was not available, the protein was modeled using a hybrid modeling server named Phyre2. The platform incorporates ab initio folding simulation called Poing 2to model regions of proteins with no detectable homology to the known structure [4-6].

Ligand preparation

Ligands expressed on NK sensitive YAC-1 cell line, viz., p30CA, p12, p15MA, p15E and NK resistant p815 cell line namely, Galectin-3, CD63 (AD1 Antigen), gp70 and p30 were studied.

The following information about p30CA (Accession no.= NP_955585.1, 263 amino acids, of Moloneymurine leukemia virus origin), p12 (Accession no.= 0804277A, 84 amino acids, of Moloneymurine leukemia virus origin), p15MA (Accession no.= NP_955583.1, 130 amino acids, of Moloneymurine leukemia virus origin), p15E (Accession no.= NP_955589.1, 196 amino acids, of Moloneymurine leukemia virus origin) and Galectin-3 (Accession no. NP_034835.1), 264 amino acids of MusMusculus origin), CD63 (Accession no. NP_001036045.1), 238 amino acids of MusMusculus origin), gp70 (Accession no. CAA41992.1), 644 amino acids of Murine Leukemia Virus, p30 (Accession no. AAA46522.1), 160 amino acids of Murine Leukemia Virus were retrieved from NCBI. The crystallized structure of above mentioned ligands were not available in RCSB Protein Data Bank , so the structure of these ligands were modeled using a hybrid modeling server named Phyre2. The platform incorporates into folding simulation called Poing2to model regions of proteins with no detectable homology to the known structure [7-10].

Molecular docking using patchdock (an automatic server for molecular docking)

Patch Dock is an algorithm used for molecular docking. Here, the input is two molecules of any type e.g., proteins, DNA, drugs. The output obtained is a list of potential complexes that are sorted by the shape complementarity criteria. The Patch Dock algorithm has been inspired by image segmentation and object recognition techniques that are used in Computer Vision. If two molecules are given, then their surfaces are divided into patches depending upon the surface shape. These patches correspond to the patterns that distinguish between the puzzle pieces. Once these patches are identified and then they can be superimposed using the shape matching algorithms (Figure 1). The algorithm has three main stages:

- Molecular Shape Representation
- Surface Patch Matching
- Filtering and Scoring

The receptor molecule along with its ligand molecule was uploaded in the PATCHDOCK server, the respective e-mail id was entered and then the results were noted (Figure 2,3).

Refining models by FIREDOCK

After running PATCHDOCK, the top 10 results were refined by using FIREDOCK. The FireDock server then addresses refinement problem of the protein-protein docking solutions. This method simultaneously targets problem of flexibility along with the scoring of solutions that are produced by fast rigid-body docking algorithms. Given up to a set of 1000 potential docking candidates, it can refine and score them based on energy function. This is the first web server that allows scoring of docking solutions and performing large-scale flexible refinement online (Figure 4,5). The results with hydrogen bonds, the D COMPLEX predict the binding affinity of the protein complex, determining the energy in kcal/mol.

RESULTS

3D structure of NK activating receptor Ly49H

The 3D structure of NK activating receptor Ly49H is not available in PDB, so it was predicted using hybrid modeling server named Phyre2 (Figure 6) [11,12].

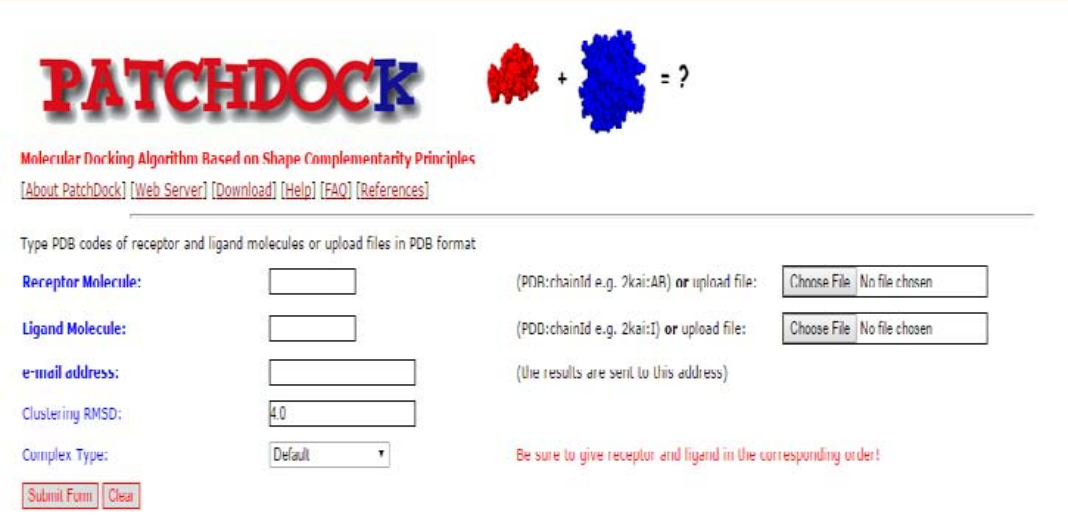
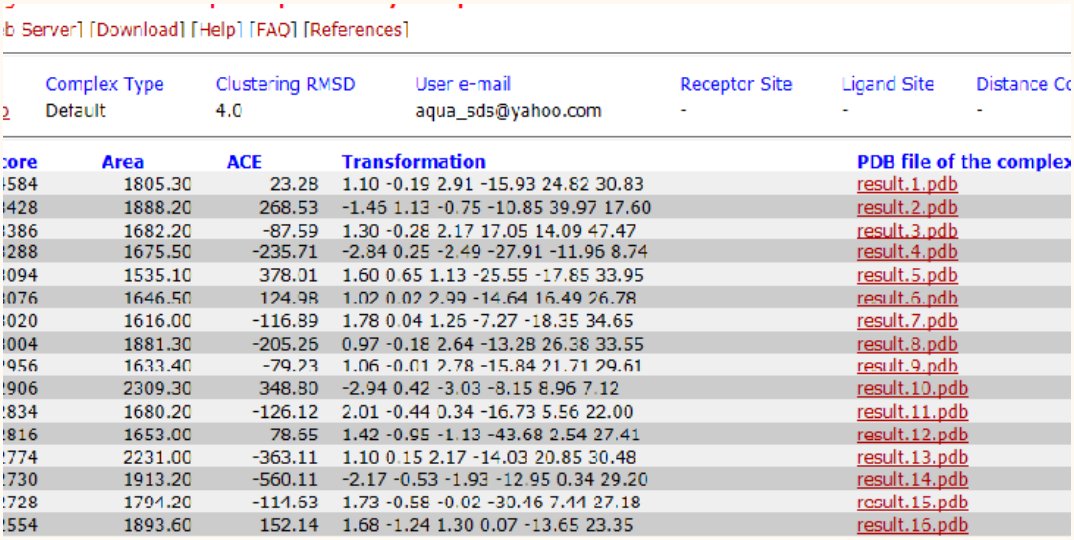


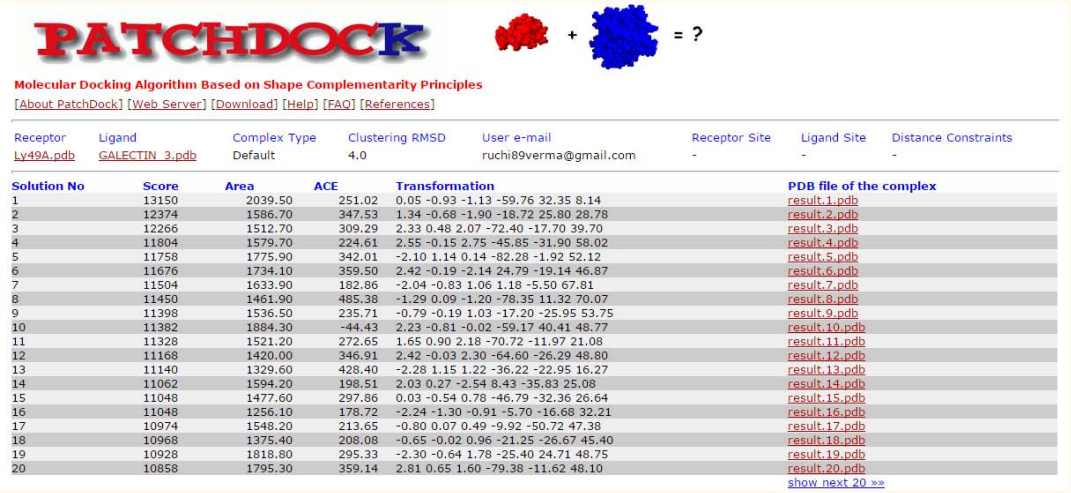
Figure 1 Screenshot of the PATCHDOCK server.



Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Co
Default	4.0	aqua_sds@yahoo.com	-	-	-

core	Area	ACE	Transformation	PDB file of the complex
584	1805.30	23.28	1.10 -0.19 2.91 -15.93 24.82 30.83	result.1.pdb
428	1888.20	268.53	-1.45 1.13 -0.75 -10.85 39.97 17.60	result.2.pdb
386	1682.20	-87.59	1.30 -0.28 2.17 17.05 14.09 47.47	result.3.pdb
288	1675.50	-235.71	-2.84 0.25 -2.49 -27.91 -11.96 8.74	result.4.pdb
094	1535.10	378.01	1.60 0.65 1.13 -25.55 -17.85 33.95	result.5.pdb
076	1646.50	124.98	1.02 0.02 2.99 -14.64 16.49 26.78	result.6.pdb
020	1616.00	-116.89	1.78 0.04 1.25 -7.27 -18.35 34.65	result.7.pdb
004	1881.30	-205.25	0.97 -0.18 2.64 -13.28 26.38 33.55	result.8.pdb
956	1633.40	-79.23	1.06 -0.01 2.78 -15.84 21.71 29.61	result.9.pdb
906	2309.30	348.80	-2.94 0.42 -3.03 -8.15 8.96 7.12	result.10.pdb
834	1680.20	-126.12	2.01 -0.44 0.34 -16.73 5.56 22.00	result.11.pdb
816	1653.00	78.55	1.42 -0.95 -1.13 -43.68 2.54 27.41	result.12.pdb
774	2231.00	-363.11	1.10 0.15 2.17 -14.03 20.85 30.48	result.13.pdb
730	1913.20	-560.11	-2.17 -0.53 -1.93 -12.95 0.34 29.20	result.14.pdb
728	1791.20	-111.53	1.73 -0.58 -0.02 -30.46 7.41 27.18	result.15.pdb
554	1893.60	152.14	1.68 -1.24 1.30 0.07 -13.65 23.35	result.16.pdb

Figure 2 Screenshot of results obtained from PATCHDOCK of Ly49H and p15E.



Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
Ly49A.pdb	GALECTIN_3.pdb	Default	4.0	ruchi89verma@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	13150	2039.50	251.02	0.05 -0.93 -1.13 -59.76 32.35 8.14	result.1.pdb
2	12374	1586.70	347.53	1.34 -0.68 -1.90 -18.72 25.80 28.78	result.2.pdb
3	12266	1512.70	309.29	2.33 0.48 2.07 -72.40 -17.70 39.70	result.3.pdb
4	11804	1579.70	224.61	2.55 -0.15 2.75 -45.85 -31.90 58.02	result.4.pdb
5	11758	1775.90	342.01	-2.10 1.14 0.14 -82.28 -1.92 52.12	result.5.pdb
6	11676	1734.10	359.50	2.42 -0.19 -2.14 24.79 -19.14 46.87	result.6.pdb
7	11504	1633.90	182.86	-2.04 -0.83 1.06 1.18 -5.50 67.81	result.7.pdb
8	11450	1461.90	485.38	-1.29 0.09 -1.20 -78.35 11.32 70.07	result.8.pdb
9	11398	1536.50	235.71	-0.79 -0.19 1.03 -17.20 -25.95 53.75	result.9.pdb
10	11382	1884.30	-44.43	2.23 -0.81 -0.02 -59.17 40.41 48.77	result.10.pdb
11	11328	1521.20	272.65	1.65 0.90 2.18 -70.72 -11.97 21.08	result.11.pdb
12	11168	1420.00	346.91	2.42 -0.03 2.30 -64.60 -26.29 48.80	result.12.pdb
13	11140	1329.60	428.40	-2.28 1.15 1.22 -36.22 -22.95 16.27	result.13.pdb
14	11062	1594.20	198.51	2.03 0.27 -2.54 8.43 -35.83 25.08	result.14.pdb
15	11048	1477.60	297.86	0.03 -0.54 0.78 -46.79 -32.36 26.64	result.15.pdb
16	11048	1256.10	178.72	-2.24 -1.30 -0.91 -5.70 -16.68 32.21	result.16.pdb
17	10974	1548.20	213.65	-0.80 0.07 0.49 -9.92 -50.72 47.38	result.17.pdb
18	10968	1375.40	208.08	-0.65 -0.02 0.96 -21.25 -26.67 45.40	result.18.pdb
19	10928	1818.80	295.33	-2.30 -0.64 1.78 -25.40 24.71 48.75	result.19.pdb
20	10858	1795.30	359.14	2.81 0.65 1.60 -79.38 -11.62 48.10	result.20.pdb

show next 20 »»

Figure 3 Screenshot of results obtained from PATCHDOCK between Ly49A with GALECTIN.

FireDock

[Web Server] [About] [Download] [FAQ] [Help] [References]

Receptor	Ligand	TransFile
ly49h.pdb	p15E.pdb	fd_trans.txt

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB	Structure show/hide
1	8	-11.92	-21.68	9.82	2.29	-2.16	☑
2	4	-8.47	-34.17	58.68	-1.56	-2.92	☐
3	7	-6.06	-7.68	5.44	-4.66	-0.57	☐
4	9	7.62	-2.54	1.53	2.06	-0.89	☐
5	3	11.36	-18.24	6.35	0.77	-1.75	☐
6	2	17.94	-10.01	5.33	8.27	0.00	☐
7	6	19.74	-10.58	5.15	4.07	-0.25	☐
8	1	23.62	-35.11	42.45	3.07	-1.89	☐
9	5	24.89	-30.24	16.98	14.02	-2.18	☐
10	10	8224.13	-64.07	10356.00	7.72	-12.47	☐

Figure 4 Screenshot of results obtained with FireDock of Ly49H and p15E.

FireDock

[Web Server] [About] [Download] [FAQ] [Help] [References]

Receptor	Ligand
Ly49A.pdb	GALECTIN_3.pdb

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB	Structure show/hide
1	9	-15.40	-28.72	12.11	8.90	-4.95	☑
2	6	-1.42	-36.54	27.59	11.61	-3.36	☐
3	7	-0.06	-3.35	1.07	-0.86	0.00	☐
4	8	7.33	-35.13	27.53	20.57	-8.51	☐
5	2	8.76	-23.75	13.63	11.73	-5.20	☐
6	4	9.15	-16.79	21.66	3.03	-1.49	☐
7	1	15.89	-10.27	3.57	8.38	-0.89	☐
8	3	24.02	-26.54	15.32	10.25	-2.07	☐
9	10	3961.24	-65.54	5059.45	-11.23	-12.77	☐
10	5	6240.62	-57.22	7917.41	-6.49	-8.90	☐

Figure 5 Screenshot of results obtained with FireDock of Ly49A with GALECTIN.

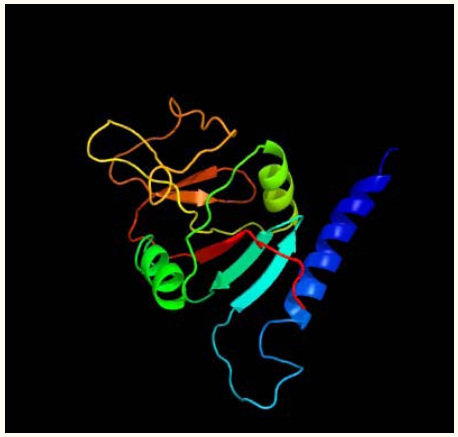


Figure 6 Modelled Crystal structure of murine Ly49H.

Structures of NK inhibitory receptor Ly49A

The crystal structure of Ly49A was not available on PDB, so the protein was modeled using a hybrid modeling server named Phyre 2 (Figure 7).

Predicted structure of YAC-1 surface ligands

For all the YAC-1 surface ligands including, p30CA, p12, p15MA, p15E, the structures were not present in PDB. So the structures were also predicted with Phyre 2 (Figure 8: A-D).

Predicted structure of P815 surface ligands

The structures of CD63, galectin-3, p30, gp70, these are the p815 surface expressed molecules were not available in PDB so, the protein structures were modeled using a hybrid modeling server named Phyre 2 (Figure 9: A-D) (Table 1).

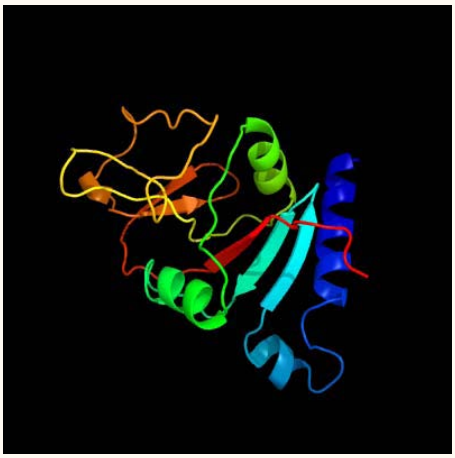


Figure 7 Predicted structure of Ly49A.

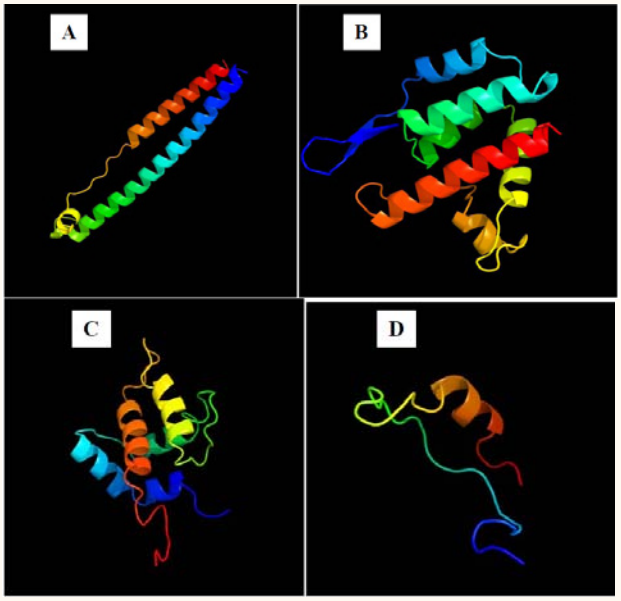


Figure 8 Modeled crystal structure of: (A) p15E, (B) p30CA, (c) p15MA, (D) p12.

Murine NK activating receptor interaction with YAC-1 surface ligands

PATCHDOCK was used to perform molecular docking Ly49H with the YAC-1 ligands; p15E, p30CA, p15MA, p12. PATCHDOCK determines top 10 complexes and for each of the complexes, the D-complex energy was calculated.

Ly49H-p15E

The results obtained after docking of receptor Ly49H with p15E were observed. For each complex obtained, following data was analyzed. Here, the complex with minimum energy is the second one with D-complex energy -12.518432Kcal/mol and the complex representing minimum energy is shown below (Figure 10) (Table 2).

Ly49H-p30CA

The complex with minimum energy is the first one as depicted in Table (3) with D-complex energy - 11.061380 Kcal/mol and the complex representing minimum energy is shown in (Table 3) (Figure 11).

Ly49H-p15MA

The complex with minimum energy is the first one as depicted in Table (4) with D-complex energy - 12.205013 Kcal/mol and the complex representing minimum energy is shown in Table (4) (Figure 12).

Ly49H-p12

The complex with minimum energy is the tenth one as depicted in Table (5) with D-complex energy - 8.455273 Kcal/mol and the complex representing minimum energy is shown in Table (5) (Figure 13).

To understand the interaction between Ly49H, activating receptor (chain-X) and the ligands (chain-Y) present (p15E, p30CA, p15MA, p12) on the surface of YAC-1 cell line, docking was performed and the D-complex energies for each receptor–ligand pair was studied. The result shows significant interaction between each of the YAC ligands with Ly49H.

MURINE NK INHIBITORY RECEPTOR INTERACTION STUDIED WITH SURFACE LIGANDS OF P815

PATCHDOCK was used to perform molecular docking of murine inhibitory surface receptor Ly49A with the surface

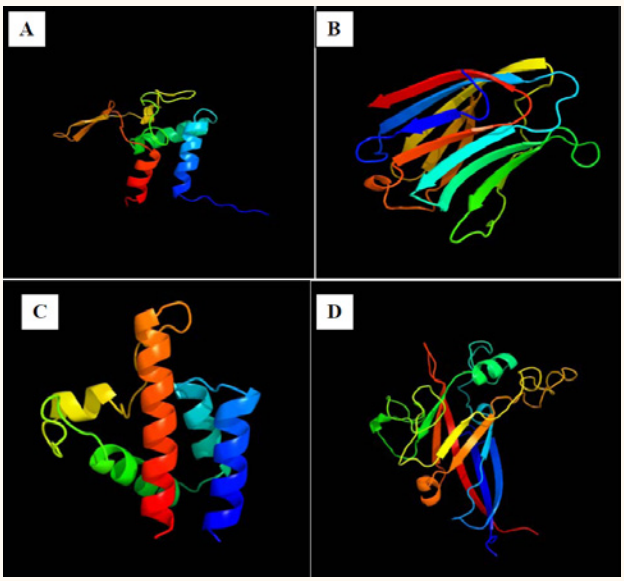


Figure 9 Predicted structure of (A) CD63, (B) Galectin -3, (C) p30, (D) gp70.

Table 1: p815 surface expressed ligands.

Ligand name	Origin	Structure availability
CD63	MusMusculus origin	Modelled using Phyre2
Galectin-3	MusMusculus origin	Modelled using Phyre2
p30	Murine Leukemia Virus	Modelled using Phyre2
gp70	Murine Leukemia Virus	Modelled using Phyre2

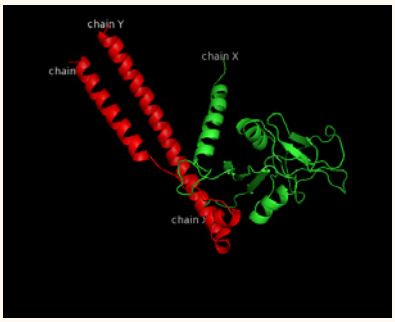


Figure 10 Complex representing minimum energy for Ly49H-p15E.

Table 2: Representing minimum energy for Ly49H-p15E.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	8	13004	1881.30	-7.958298
2	4	13288	1675.50	-12.518432
3	7	13020	1616.00	-6.078592
4	9	12956	1633.40	-6.998742
5	3	13386	1682.20	-3.906250
6	2	13428	1888.20	-6.315068
7	6	13076	1646.50	-6.355111
8	1	14584	1805.30	-11.026176
9	5	13094	1535.10	8.240053
10	10	12906	2309.30	-6.856760

Table 3: Representing minimum energy Ly49H-p30CA.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	10	12816	1782.60	-11.061380
2	1	15552	2125.10	-11.043210
3	3	13640	2610.20	3.583099
4	9	12842	1828.80	-6.233670
5	8	12864	1538.10	-10.211391
6	2	14556	1712.60	-10.105183
7	7	12866	1910.70	-6.869576
8	6	13456	2312.60	-4.854557
9	5	13596	1979.00	-8.113094
10	4	13596	2295.70	-4.700000

ligands of p815 (CD63, Galectin-3, p30, gp70). PATCHDOCK determines top 10 complexes and for each of the complex, the d-complex energy was calculated

Ly49A- CD63

The results obtained after docking of receptor Ly49A with CD63 were observed. For each complex obtained, following data analyzed (Table 6) (Figure 14).

Here, the complex with minimum energy is the first one with D-complex energy -6.579559 Kcal/mol and the complex representing minimum energy is shown above.

Ly49A-Galectin-3

The results obtained after docking of receptor Ly49A with Galectin-3 were observed. For each complex obtained, following data was analyzed (Table 7) (Figure 15).

Here, the complex with minimum energy is the eight one with D-complex energy-10.283447Kcal/mol and the complex representing minimum energy is shown above

Ly49A-p30

The results obtained after docking of receptor Ly49A with p30 were observed. For each complex obtained, following data was analyzed.

Here, the complex with minimum energy is the second one

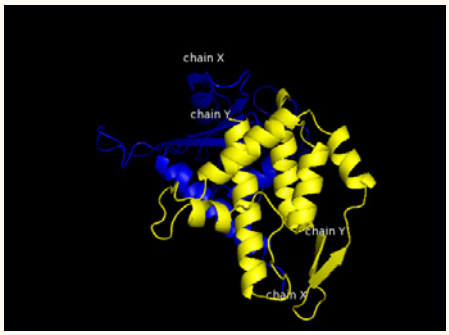


Figure 11 Complex representing minimum energy Ly49H-p30CA.

Table 4: Representing minimum energy Ly49H-p30CA.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	**3**	**13172**	**1629.80**	**-12.205013**
2	8	12380	1610.70	-7.548349
3	9	12376	1735.30	-9.758832
4	10	12344	2049.90	-3.523870
5	4	12922	2142.90	-10.070923
6	1	13532	2317.30	-3.179320
7	6	12582	2069.70	-4.670235
8	5	12630	1824.10	-4.355385
9	2	13396	1937.70	10.206377
10	7	12444	1942.00	-4.854555

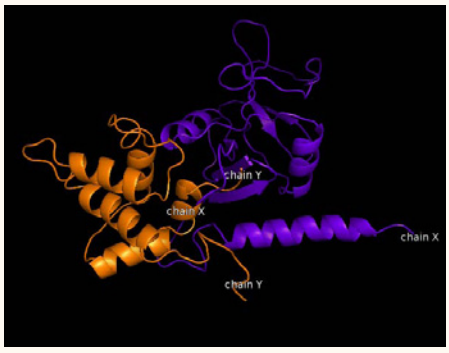


Figure 12 Complex representing minimum energy for Ly49H-p15MA.

Table 5: Representing minimum energy Ly49H-p12.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	4	11670	1548.10	-7.701501
2	7	11378	1659.40	-8.169250
3	10	11220	1631.30	-4.092175
4	2	12534	1759.70	-8.218939
5	9	11258	1641.80	-6.653870
6	5	11626	1527.90	-5.149787
7	3	11872	1649.40	-3.852422
8	1	12808	1783.50	-4.707519
9	8	11366	1673.60	-6.188818
10	6	11378	1796.90	-8.455273

Figure 13 Complex representing minimum energy Ly49H-p12.

Table 6: D-complex energies for Ly49A forLy49A-CD63.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	1	14340	2093.20	-6.579559
2	2	14216	2164.10	-4.585131
3	9	12984	2185.50	-4.631744
4	3	13956	1819.70	-5.695937
5	7	13122	2285.50	-4.700000
6	4	13836	1727.90	-5.003655
7	8	13106	2010.20	-5.607669
8	10	12824	1805.30	-3.485970
9	6	13400	2101.20	-1.997861
10	5	13614	2330.20	-4.003188

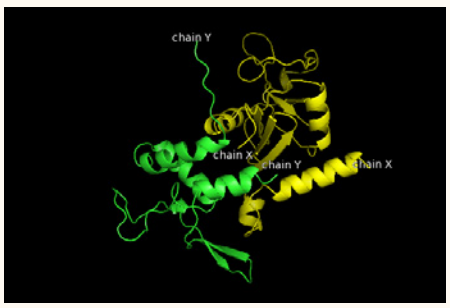


Figure 14 Complex representing minimum energy.

Table 7: D-complex energies for Ly49A-galectin-3.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	9	11398	1536.50	-9.467794
2	6	11676	1734.10	-6.930770
3	7	11504	1633.90	111.539484
4	8	11450	1461.90	-8.500221
5	2	12374	1586.70	-5.118127
6	4	11804	1579.70	-6.278455
7	1	13150	2039.50	-7.149738
8	3	12266	1512.70	-10.283447
9	10	11382	1884.30	-4.452348
10	5	11758	1775.90	-4.745952

Figure 15 Complex representing minimum energy for Ly49A-Galectin-3.

with D-complex energy-11.074407 Kcal/mol and the complex representing minimum energy is shown in Table (8) Figure (16).

Ly49A-gp70

The results obtained after docking of receptor Ly49A with gp70 were observed. For each complex obtained, following data was analyzed (Table 9) (Figure 17).

Here, the complex with minimum energy is the first one with D-complex energy -11.879547 Kcal/mol and the complex representing minimum energy is shown above.

DISCUSSION

The evasion of tumors in spite of host immune defenses is by the interaction of various tumor ligands with their receptors on immune cells that affects the function of host cells involved in immune responses. So, different tumor-derived factors or ligands may affect the function of natural killer (NK) cells by up-regulatory and down-regulatory modulation of receptors [4].

The present study aimed at identifying the multiple factors derived from NK sensitive cell line (YAC-1) and NK resistant cell line (P815) that might be involved in bringing about changes in the NK cytotoxicity outcome. Ly49H is a prototypic activating receptor while Ly49A has been taken as a prototypic inhibitory receptors found in mouse NK cells. Sequences of respective ligands from YAC-1 and P815 cells were obtained from NCBI. Further, PATCHDOCK was used to perform molecular docking of Ly49H with the YAC-1 ligands and Ly49A with P815 ligands.

Table 8: D-complex energies for Ly49A-p30.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	7	11666	1424.20	-7.158205
2	**2**	**12416**	**1508.90**	**-11.074407**
3	8	11572	1458.00	-6.778593
4	6	11796	1786.60	24.503307
5	3	12182	2217.50	-2.055967
6	1	12634	2205.30	-4.072277
7	4	11902	1621.60	-4.700000
8	5	11842	1753.60	-4.519311
9	9	11508	1378.70	-5.943563
10	10	11504	1664.90	-4.636067

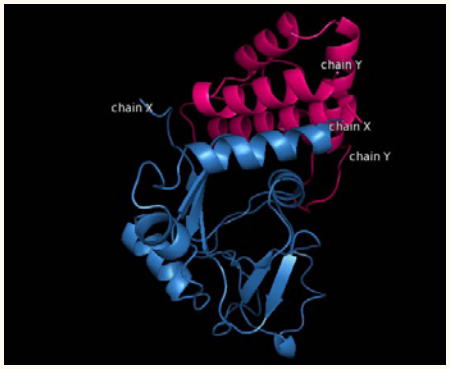


Figure 16 Complex representing minimum energy for Ly49A-p30.

Table 9: D-complex energies for Ly49A-gp7.

Rank	Solution no.	Score	Area	D-complex (kcal/mol)
1	**4**	**13626**	**2245.00**	**-11.879547**
2	1	14438	1899.30	-3268851
3	8	13022	1804.90	-8.566955
4	2	13964	1872.50	-5.879165
5	7	13092	1835.20	-5.508979
6	6	13238	1989.30	-8.161250
7	5	13384	1838.60	-3.141820
8	9	12904	1721.20	-4.220493
9	3	13920	2097.80	-5.034545
10	10	12840	2833.60	9.995013

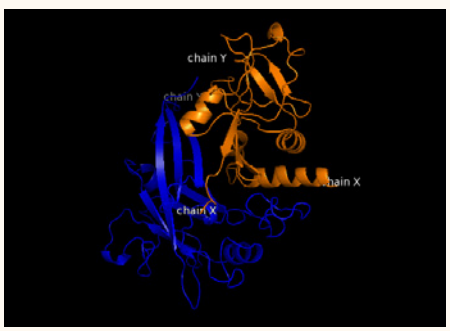


Figure 17 Complex representing minimum energy for Ly49A-gp70.

PATCHDOCK determines top 10 complexes for each of the ligand-receptor complexes from which the D-complex energy was calculated.

The ligands expressed by YAC tumor cells p30CA,p12,p15MA and p15E show significant binding efficiency with Ly49H and the ligands expressed by P815 cells line galactin-3,CD63 (AD1 antigen),gp70 and p30show significant binding efficiency with Ly49A.

Our study provides direct evidence that in case of YAC1 cell line which was known to show susceptibility to NK cytotoxicity owing to its low level of expression of MHC I thus preventing engagement of inhibitory receptors on NK cells is not the only reason for its susceptibility. Yac1 cell expressed ligands, p30CA, p12, p15MA and p15E all show significant binding to activating NK cell receptor thus leading to activation signal transduction for cytotoxicity.

P815 cell line known to be resistant to NK cell mediated cytotoxicity has been known to express high levels of MHCI and hence engage inhibitory receptors on NK cells resulting in escape from NK mediated killing. However, in the present study, we have also shown that P815 expressed ligands galactin-3,CD63 (AD1 antigen),gp70 and p30, all show direct significant binding to inhibitory Ly 49 receptor thus providing direct evidence that engagement of tumor expressed ligands by inhibitory receptors are significant in providing resistance of P815 from NK mediated cell lysis.

CONCLUSION

Killing of tumor cells by NK cells is a dynamic interaction and signaling process in which binding of activating ligands to NK activating receptors induce target cell mediated killing by initiating cascade of signaling reactions resulting in cytolysis by NK cells. Susceptibility of tumor cells to NK may often be due to reduced engagement of inhibitory receptors by down regulation of MHC I ligand for NK inhibitory receptors. However in case of YAC-1 cells, we have shown conclusively that it is not only down regulation of inhibitory signal that results in activation but direct engagement of activating receptors by tumor expressed ligands on YAC-1 cells results in upregulation of activation signals in NK resulting in susceptibility of YAC cells to NK mediated cytotoxicity. In case of NK resistant cell line, P815 engagement of specific tumor expressed ligands by inhibitory receptors in NK cells results in interference of the activating signaling cascade resulting in target cell being spared by NK cells. Each NK cell expresses a multitude of activating and inhibitory receptors. The sum total of these opposing signals results in the outcome of the NK target interaction.

In the present study, surface expressed molecules present on p815 and YAC-1 cells were studied for binding with inhibitory receptors and activating receptors respectively. The results obtained show that the binding affinities obtained with docking of murine NK inhibitory receptor Ly49A with the surface ligands of mastocytoma p815 were quite significant (CD63 with -6.579559, galectin-3 having -10.283447, p30 with -11.074407 and gp70 having -11.879547 binding affinities) this tumor is itself known to modulate NK by changing the receptor profile of it and hence, further increasing the inhibition. Multiple inhibitory

ligands were engaged by p815 cells and further studies need to be carried out whether these ligands of NK resistant cell line cause modulations in inhibitory receptor profiling, while docking results of NK activating receptor Ly49H with the surface ligands of T-lymphoma YAC-1 were also significantly marked (p15E with -12.518432, p30CA with -11.061380, p15MA with -12.205013 and p12 with -8.455272), which activate the NK effector function and tumor cells could not resist themselves anymore and thus are killed.

In the same way, other important surface proteins can be taken in future studies like Moloney leukemia virus-determined cell surface antigen (MCSA) [5], NKTS [6], p71 [5,6,13-15], H-2a , etc., once their sequences are deduced.

In the present studies we tried to elucidate the molecular basis of susceptibility of YAC tumor cells and resistance of P815 cells to NK mediated cytotoxicity.

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REFERENCES

1. Zucchini N, Crozat K, Baranek T, Robbins SH, Altfeld M, Dalod M. Natural killer cells in immunodefense against infective agents. Expert Rev Anti Infect Ther. 2008; 6: 867-885.
2. Jonjić S, Babić M, Polić B, Krmpotić A. Immune evasion of natural killer cells by viruses. Curr Opin Immunol. 2008; 20: 30-38.
3. Roder JC, Kiessling R, Biberfeld P, Andersson B. Target-effector interaction in the natural killer (NK) cell system. II. The isolation of NK cells and studies on the mechanism of killing. J Immunol. 1978; 121: 2509-2517.
4. Nelson DS, Nelson M. Evasion of host defences by tumours. Immunol Cell Biol. 1987; 65: 287-304.
5. Troy FA, Fenyg EM, Klein G. Moloney leukemia virus-induced cell surface antigen: Detection and characterization in sodium dodecyl sulfate gels. Proc Nati Acad Sci USA. 1977; 74: 5270-5274.
6. Roder JC, Rosén A, Fenyö EM, Troy FA. Target-effector interaction in the natural killer cell system: isolation of target structures. Proc Natl Acad Sci USA. 1979; 76: 1405-1409.
7. Cortegano I, del Pozo V, Cárdaba B, de Andrés B, Gallardo S, del Amo A, et al. Galectin-3 down-regulates IL-5 gene expression on different cell types. J Immunol. 1998; 161: 385-389.
8. Godin-Ethier J, Hanafi LA, Piccirillo CA, Lapointe R. Indoleamine 2, 3-dioxygenase expression in human cancers: clinical and immunologic perspectives. Clin Cancer Res. 2011; 17: 6985-6991.
9. Dorfman JR, Raulet DH. Acquisition of Ly49 receptor expression by developing natural killer cells. J Exp Med. 1998; 187: 609-618.
10. John CM, Leffler H, Kahl-Knutsson B, Svensson I, Jarvis GA. Truncated galectin-3 inhibits tumor growth and metastasis in orthotopic nude mouse model of human breast cancer. Clin Cancer Res. 2003; 9: 2374-2383.
11. Long EO. Regulation of immune responses through inhibitory receptors. Annu Rev Immunol. 1999; 17: 875-904.
12. Lopez DM, Lopez-Cepero M, Watson GA, Ganju A, Sotomayor E, Fu YX. Modulation of the immune system by mammary tumor-derived factors. Cancer Invest. 1991; 9: 643-653.
13. Moretta A, Biassoni R, Bottino C, Mingari MC, Moretta L. Natural cytotoxicity receptors that trigger human NK-cell-mediated cytolysis. Immunol Today. 2000; 21: 228-234.
14. Al-Rammahy AK, Levy JG, Jackson AL. Isolation and partial characterization of a tumor-associated antigen of the P815 mastocytoma of DBA/2 mice. J Immunol. 1980; 124: 143-150.
15. Roder JC, Ahrlund-Richter L, Jondal M. Target-effector interaction in the human and murine natural killer system: specificity and xenogeneic reactivity of the solubilized natural killer-target structure complex and its loss in a somatic cell hybrid. J Exp Med. 1979; 150: 471-481.

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