

## CONTENTS

<b>TOPIC</b>	<b>Page No.</b>
<i>List of figures</i>	<i>i-ii</i>
<i>List of tables</i>	<i>iii-iv</i>
<i>List of abbreviations</i>	<i>v-vi</i>
<b>1. ABSTRACT</b>	<b>1</b>
<b>2. INTRODUCTION</b>	<b>2-4</b>
<i>2.1 NK cell receptors</i>	<b>3</b>
<i>2.2 Tumor cell lines and cell culture</i>	<b>3</b>
<i>2.3 Cell culture</i>	<b>4</b>
<i>2.4 Primary culture</i>	<b>4</b>
<b>3. LITERATURE SURVEY</b>	<b>5-16</b>
<i>3.1 NK receptors</i>	<b>5</b>
<i>3.2 Balance between activating and inhibitory signals</i>	<b>7</b>
<i>3.3 NK cell activation and cytotoxicity</i>	<b>8</b>
<i>3.4 NK cell activating receptor and ligands interaction</i>	<b>8</b>
<i>3.5 Natural cytotoxicity receptors (NCRs) and their still subtle cellular ligands</i>	<b>9</b>
<i>3.6 Tumors, tumour derived factors and NK cell response</i>	<b>9-13</b>
<i>3.7 Other tumor derived factors</i>	<b>13-16</b>
<b>4. MATERIALS AND METHODS</b>	<b>17-30</b>
<i>4.1 Receptor Modelling</i>	<b>17</b>
<i>4.2 Ligand Preparation</i>	<b>17</b>
<i>4.3 Molecular Docking using PATCHDOCK (an automatic server for molecular docking)</i>	<b>17-18</b>

4.4 Refining models by FIREDOCK	18
4.5 Tumor cell lines - YAC-1 (NK-sensitive tumor cell line) cell line features	19-20
4.6 Culture media	20
4.7 SDS-PAGE electrophoresis (laemmli's method, 1970) buffers	21
4.7.1 Stock solutions	22
4.7.1.1 Solution a- 30% acrylamide solution	22
4.7.1.2 Solution b- 1.5 m tris buffer (pH-8)	22
4.7.1.3 Solution c- 0.5 m tris buffer(pH-6.8)	22
4.7.1.4 Solution d- 10% APS	22
4.7.2 Composition of gel solution for a 1mm thick gel	22
4.7.3 Stock solution-10% SDS solution	22
4.7.4 Sample solubilizing buffer (SSB) (1x)	22
4.7.5 Electrophoretic buffer	23
4.7.6 Staining solution	23
4.7.7 Destaining solution	23
4.7.8 Silver staining reagents (for 300ml)	23
4.8 Phosphate saline buffer composition (pH: 7.3 -7.4)	24
4.9 Whole cell lysis buffer (for 30 ml)	24
4.10 Membrane fractionation buffers	25
4.11 Methods in animal cell culture (YAC-1 cell line)	25
a) Splitting of cells	25
b) Freezing of cells	25
c) Thawing of cells	25-26
4.12 Cell number determination using Neubauer chamber	26
4.12.1 Counting through hemocytometer	26
4.12.2 Procedure to count cells	26
4.12.3 Cell viability assay	27
4.13 Collection of YAC-1 supernatant	27
4.14 Generation of growth curve of NK cell susceptible cell line – YAC-1	27
4.15 Method for cell lysate preparation	28
4.16 Method for whole membrane extraction	28
4.17 Characterisation of protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	28-30
<b>5. RESULTS</b>	<b>31-40</b>
5.1 Structures of NK activating receptor Ly49H	31

<i>5.2 Predicted structures of ligands</i>	<b>32</b>
<i>5.3 Murine NK activating receptor interaction with YAC-1 surface ligands</i>	<b>33</b>
<i>5.3.1 ly49H-p15E</i>	<b>33</b>
<i>5.3.2 Ly49H-p30CA</i>	<b>34</b>
<i>5.3.3 Ly49H-p15MA</i>	<b>35</b>
<i>5.3.4 Ly49h-p12</i>	<b>36</b>
<i>5.4 Characterization of tumor cell line (YAC-1) to study the tumor derived factors that may induce NK cell modulation</i>	<b>37</b>
<i>5.5 Generation of growth curve of NK cell susceptible cell line, YAC-1</i>	<b>38</b>
<i>5.6 Characterization of tumor cell line (YAC-1) to study the tumor lysate that may induce NK cell modulation</i>	<b>39</b>
<i>5.7 Characterization of tumor cell line (YAC-1) to study the tumor lysate and whole membrane protein that may induce NK cell modulation</i>	<b>40</b>
<b>6. DISCUSSION AND FUTURE PERSPECTIVE</b>	<b>41-42</b>
<b>7. CONCLUSION</b>	<b>43</b>
<b>8. REFERENCES</b>	<b>44-51</b>