Regulation of Expression of NK cell Receptor by Tumour derived Transcription Factor

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OF

MASTER OF TECHNOLOGY

IN

BIOINFORMATICS

SUBMITTED BY

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(2K16/BIO/09)

UNDER THE SUPERVISION

OF

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CANDIDATE'S DECLARATION

I, **Vikrant Khokhar**, Roll no. 2K16/BIO/09, student of **M.Tech (Bioinformatics)**, hereby declare that the project Dissertation titled "**Regulation of Expression of NK cell Receptors by Tumour derived Transcription Factors**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi

Date:

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M.Tech. (Bioinformatics)

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CERTIFICATE

I, hereby certify that the Project Dissertation titled "**Regulation of Expression of NK cell receptors by Tumour derived transcription Factors**" which is submitted by **Vikrant Khokhar**, Roll no. 2K16/BIO/09, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

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ABSTRACT

Natural killer (NK) cells were identified 30 years ago based on their ability to "spontaneously" kill tumour cells. The NK cell recognition and activation is due to presence of receptors that binds to specific ligands on tumour cells and normal cells. These receptors have the ability to modulate and activate NK cell function. NK cell response is regulated by the balance between the signals by activating and inhibitory receptors. The outcome of immune response is determined by the extent of strength of activating and inhibitory signals. The expressions of inhibitory receptor were found to be up regulating and activating receptor is down regulating against tumour cell. Tumour cell had reported in escape NK mediated recognition by down regulating the expression of ligands for activating receptors and over expressing the ligand for inhibitory receptors. NK cell inhibitory function can be a means for promotion and regression of tumour and exploring means of blockade of NK cell inhibitory receptors is a way to promote immune response against tumour. Cancer therapies are being developed based on preventing NK cell inhibition or activation of NK cell receptors and modulation of T cell function. Transcription factors (TFs) are key molecules in the regulation of gene transcription and have a significant influence on immune cells growth and development. Many primary and modified genes leading to cancer, participate in many pathways of NK cell development and maturation. Tumours are essentially tissues that have overcome normal regulation mechanisms, and therefore the ability to distinguish normal cells from abnormal cells is a key part of selectively attacking tumour cells. TF derived from tumour cells like GATA -3, ER β, Helios A, bind on the 5'UTR region of NK cell inhibitory receptor genes and modulate their normal regulation by affecting their signaling pathways. These tumour derived TF up regulate and down regulate the signaling of NK cell and abnormalities in signaling pathway leads to progression of tumour cells. Understanding the NK cell receptors and their recognition mechanisms provides new ways for the development of immunotherapies against cancer.

ACKNOWLEDGEMENT

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I would also like to extend my heartfelt gratitude towards all the members of Immunoinformatics lab for gratuitously helping me in the successful completion of the project.

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LIST OF ABBREVIATIONS

AHR	Aryl hydrocarbon Receptor
APC	Adenomatous Polyposis Coli
APO	Apoptosis Antigen One
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator
ATF2	Activating Transcription Factor 2
BAD	Bcl-2 Associated agonist of cell death
BAX	Bcl-2 Associated X protein
BCL	B-cell Lymphoma
BLAST	Basic local Alignment Search tool
CD	Cluster of Differentiation
cDNA	Complementary DNA
DNA	Deoxyribonucleic Acid
ER	Estrogen Receptor
EGFR	Epidermal Growth Factor receptor
ERK	Extracellular signal Regulated Kinase
FAS	First Apoptosis Signal Receptor
GADD	Growth Arrest and DNA Damage
HLA	Human Leukocyte Antigen
Hsp	Heat-shock protein
HTML	Hypertext Markup Language
IL	Interleukin
IFN	Interferon
KIR	Killer cell immunoglobulin-like Receptor
LIR	Leukocyte Inhibitory Receptor
MAP	Mitogen Activated Protein
MHC	Major Histocompatibility Complex
NCBI	National centre for Biotechnology Information
NCR	Natural Cytotoxicity Receptor
Nfatc	Nuclear factor of Activated T-cell

NK	Natural-killer Cell
PCNA	Proliferating Cell Nuclear Antigen
PWM	Positional Weight Matrix
Rb	Retinoblastoma
RAR	Retinoic Acid Receptor
RXR	Retinoid X Receptor
TBP	TATA Binding Protein
TF	Transcription Factor
TFBS	Transcription Factor Binding Site
TP	Tumour Protein
TRANSFAC	Transcription factor database
UTR	Un-translated region
XML	eXtensible Markup Language

CHAPTER 1 INTRODUCTION

1.1 OVERVIEW OF NK CELL

Natural killer cell is a type of lymphocyte (a white blood cell) and a component of innate immune system. NK cells play a major role in the host-rejection of both tumours and virally infected cells. Natural killer cells are large granular lymphocyte, they are non-phagocytic cells and the granules of NK cells contain pre formed biologically potent molecules (Trinchieri, 1989). Some of these molecules have capability to form pores in the membrane of target cells resulting in the lysis of the cell and some other molecules induce apoptosis in the target cell. NK cell play important role in defense against both intracellular and extracellular pathogen (Moretta et al., 2002). When a cell is infected with virus, then cell secrete type 1 interferon, these interferon bind to the interferon receptors on the NK cell and as a result NK cell get activated and they proliferated and differentiate into effector NK cell. This effector NK cells has capability to recognize the cells which are infected by viruses for this the use specific receptors present on the surface which recognize the altered protein on the surface of infected cell. NK cells makes contact with the infected cells through these receptors, once contact has been established the granules of the effector NK cells diffuse with the plasma membrane of NK cell and these granules are reached on to the outer surface of the target cell, these granule can contain biologically potent molecules most of which are toxic proteins also known as cytotoxins, one of the example is perforin, that entered into the plasma membrane of the target cell and create perforation in the membrane this results in the lysis of the cell or cytolysis (Luevano, Madrigal, & Saudemont, 2012) . Another important toxic protein is granzyme. Granzyme induce the target cell to undergo apoptosis thus NK Cell responsible for the killing of most infected cells either by cytolysis or by apoptosis or programmed cell death.

Now understand how NK cells provide defense against extracellular pathogens, we know NK cells are non phagocytic cell so they cannot kill extracellular pathogens directly, NK cells secrete interferon gamma that is type 2 interferon, this interferon acts as macrophage activation factor,

these interferons once released by the natural killer cells bind to the specific receptors on the macrophages as a result macrophages become more activated and more efficient in phagocytosis and destruction of the pathogens. Natural killer cells provide defense against both intracellular and extracellular pathogens, in case of intracellular pathogens T-cells are directly involved but they provide defense against extracellular pathogen indirectly. NK cells play a major role in the host-rejection of both tumours and virally infected cells.

1.2 Types of NK cell receptors

1.2.1 Activating receptors

- Ly49 (homodimers), relatively ancient, C-type lectin family receptors, are of multigenic presence in mice, while humans have only one pseudogenicLy49, the receptor for classical (polymorphic) MHC I molecules.
- NCR (natural cytotoxicity receptors), a type of type 1 transmembrane proteins of the immunoglobulin super family, upon stimulation, mediate NK killing and release of IFNγ. They bind viral ligands such as hemagglutinins and hemagglutinin neuraminidases, some bacterial ligands and cellular ligands related with tumour growth such as PCNA.

1.2.2 Inhibitory receptors

Killer-cell immunoglobulin-like receptors (KIRs) belong to a multigene family of more recently evolved Ig-like extracellular domain receptors; they are present in nonhuman primates, and are the main receptors for both classical MHC I (HLA-A, HLA-B, HLA-C) and nonclassical Mamu-G (HLA-G) in primates. Some KIRs are specific for certain HLA subtypes. Most KIRs

are inhibitory and dominant. Regular cells express MHC class 1, so are recognized by KIR receptors and NK cell killing is inhibited.

- CD94/NKG2 (heterodimers), a C-type lectin family receptor, is conserved in both rodents and primates and identifies nonclassical (also nonpolymorphic) MHC I molecules such as HLA-E. Expression of HLA-E at the cell surface is dependent on the presence of nonamer peptide epitope derived from the signal sequence of classical MHC class I molecules, which is generated by the sequential action of signal peptide peptidase and the proteasome. Though indirect, this is a way to survey the levels of classical (polymorphic) HLA molecules.

- ILT or LIR (leukocyte inhibitory receptors) — are recently discovered members of the Ig receptor family.

- Ly49 (homodimers) have both activating and inhibitory isoforms. They are highly polymorphic on the population level; though they are structurally unrelated to KIRs, they are the functional homologues of KIRs in mice, including the expression pattern. Ly49s are receptor for classical (polymorphic) MHC I molecules

1.3 OVERVIEW OF TRANSCRIPTION FACTOR

The first step in gene expression is transcription which results in formation of primary RNA transcript from DNA. This is very critical step and leads to formation of protein. Basal transcription and its regulation depends on some factors and these factors are known as transcription factor. These factors bind to DNA sequence and control transcription of that particular gene. The TF are classified into families on the basis of precise protein structure that are used to mediate binding to DNA or to cause factor dimerization which is useful for DNA binding.

1.3.1 Activity of Transcription Factor

Activation

The ability of a transcription factor to activate transcription has been shown to be dependent upon specific regions of the protein which are distinct from the region mediating DNA binding and are known as activation domains (Ptashne 1988). Various types of activation domains are present that are rich in acidic amino acids, proline residues or glutamine residues. These activation domains can interact with other proteins factors or RNA polymerase enzyme itself (Sigler 1988). The important contact factor is known as TATA binding protein.

Repression

Most of the TF are work in positive manner but they also work in inhibiton of transcription. For example in β-interferon promoter where the binding of two positively acting factors is necessary for gene activation but another factor acts in negative manner and it binds to DNA region and prevent the binding of positively acting factor. Another way that an inhibitory transcription factor can act is by interfering with the activation of transcription mediated by a bound factor in a phenomenon known as quenching (Keleher et a/. 1988).

1.3.2 Regulation of Transcription

For transcription to be regulated, some modulation is present for the activity of specific factors that they produce correct pattern of gene expression. This modulation is achieved either by regulating the synthesis of the particular transcription factor so that it is present only in a specific cell type, or by regulating its activity so that it is present in an active form only in a specific cell type or by ensuring that activation occurs only in response to a specific signal.

Regulation of synthesis

Examples of the role of regulated synthesis in controlling tissue specific gene transcription are Oct-2 and MyoD transcription factors. These factors involved in the stimulation of Ig gene expression in B cell and the latter is expressed in those genes that encodes creatine kinase and these genes are expressed in muscle cells. Oct-2 is present in B-cells but absent from cell types like HeLa cells that do not express Ig genes (Scheidereit et a/. 1987). The expression of the gene encoding Oct-2 in HeLa cells results in the transcriptional activation of immunoglobulin genes introduced into these cells.

Regulation of activity

Although regulation of transcription factor synthesis is widely used as a method of gene regulation, it suffers from the deficiency that the transcription of the genes encoding the transcription factors themselves must be regulated. Hence it only sets the problem one step back. A simple example of such modulation occurs in the yeast transcription factor ACE1. This factor activates transcription of the metallothionein gene in response to copper. In this case the protein undergoes a conformational change in the presence of copper which allows it to bind to regulatory sites in the metallothionein gene and activate transcription (Furst et a/. 1988; Figure 3a).

1.4 Role of transcription factor in tumourigenesis

Transcription factor play very important role in expression of the particular gene. Cancer indicates the uncontrolled proliferation of a particular cell. Which can be caused by unwanted mutation and as a result defects in many class of genes (Luevano et al., 2012). There were many genes that govern cell division and responsible for cancer, and well known class of genes are proto-oncogene (gain of function) that enhance or activate the cell cycle and tumour suppressor gene (loss of function) that slow down or stop the growth of the cells. Transcription factor

participate in many signaling pathway and regulate their signaling by up regulate or down regulate the rate of transcription so gene product may or may not be responsible for the abnormalities in signaling pathways .

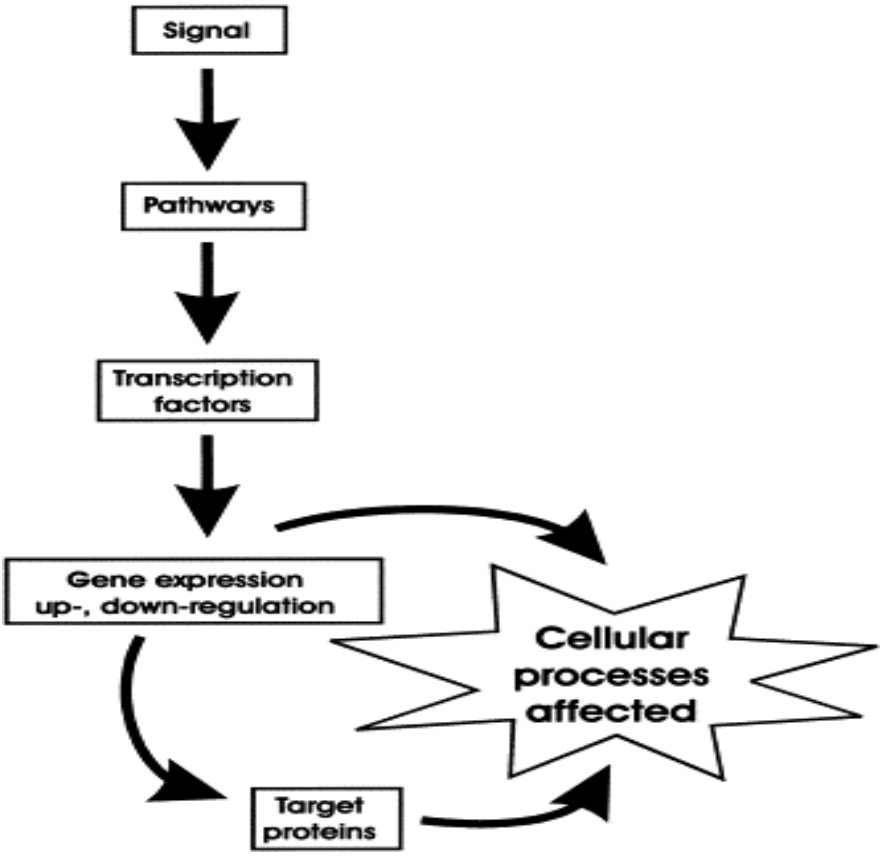

Figure1. Effect of TF on Cellular processes

Chapter 2 REVIEW OF LITERATURE

The term NK cell is assigned on the basis of NK cell function these are the lymphoid cells having ability to lysis tumour cell without any stimulation. We know that the origin of NK cell and T-cell is same that is lymphoid progenote. We can obtain NK cell in vitro from cd34 cells which were isolated from umbilical cord blood, bone marrow and from thymus in the presence of appropriate cells or IL-15 (Vosshenrich etal. 2006). NK cells having ability to discriminate between normal and tumour cell, predicted by missing self hypothesis. NK cell recognize MHC class 1 molecule present on the cell membrane, through surface receptor as a result delivering signal that inhibit instead of activation of NK cells. If the NK cells recognize a target cell with low expression or insufficient expression of MHC class 1 molecule are occur in tumour cell or viral infected cell then the NK cell lyses the target cell [Blume-Jensen et al., 2001; Darnell, 2002].

Some example of transcription factor that regulate the signaling of NK cells, TCF1 and Groucho (Fig. 3); the ER, AHR, ARNT, RAR and RXR (Fig. 4); JUN, FOS, ATF2, ELK1, GADD153, c-ABL, NFB, IB, RB1, E2F, TBP, TP53 and TP21 at the end of the MAP kinase cascade (Fig. 5) (Khanna and Jackson, 2001); BAX, FAS, APO1, BAD, BCL2, GADD45 (Nebert, 2002).

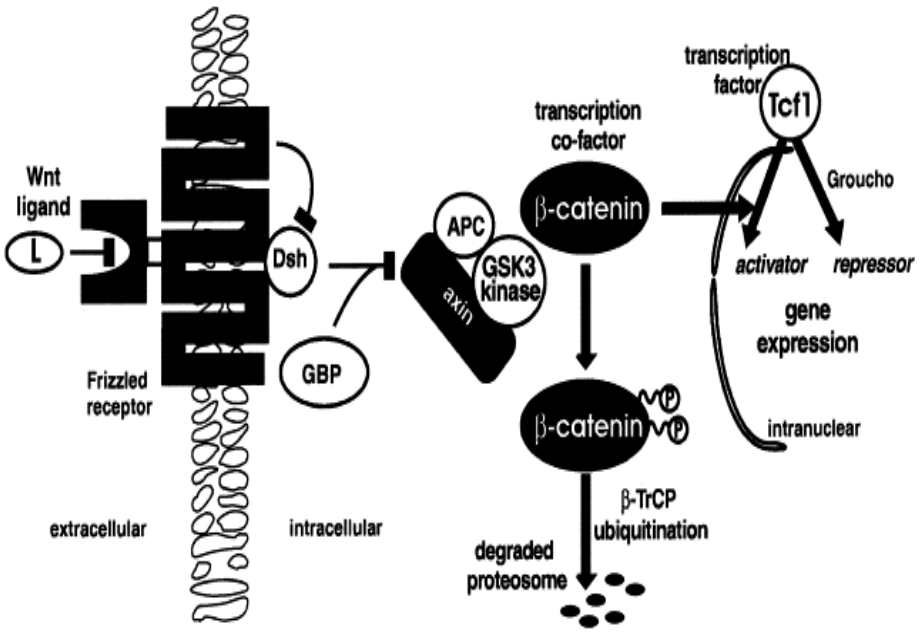

Figure2. Diagrammatic representation of Wnt – catenin signal. As an example of how this pathway might be associated with tumourigenesis, APC (adenomatous polyposis coli) is a tumor suppressor gene, and APC malfunction leads to chromosomal instability (Livingston, 2001). Wnt signaling pathways are known to be involved in cancer (Taipale and Beachy, 2001).

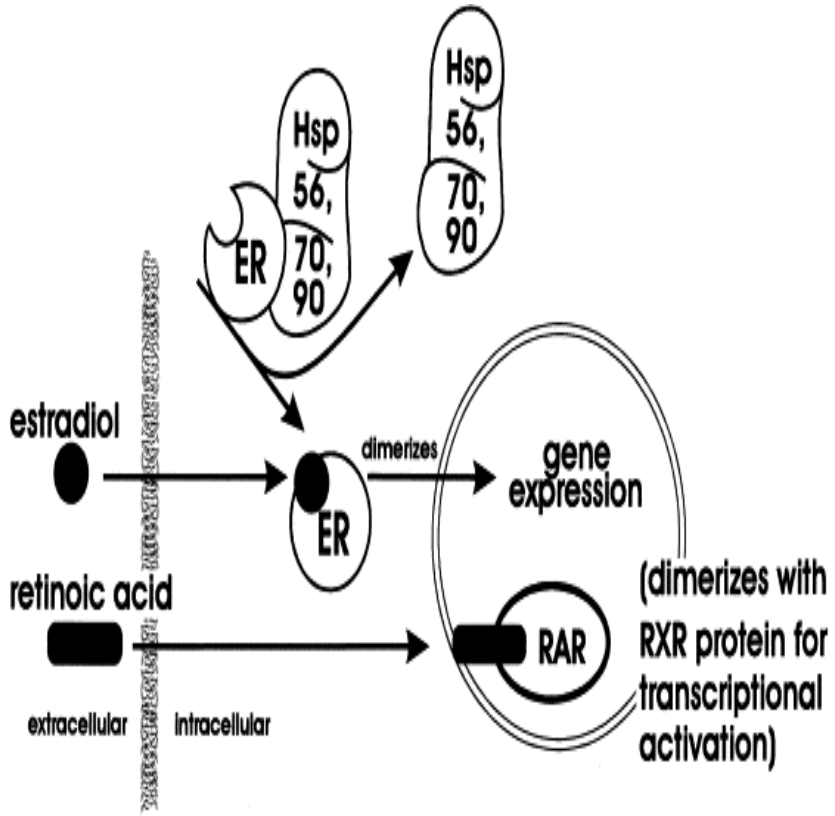

Figure3. Illustration of the nuclear hormone signal transduction pathway with the cytoplasmic estrogen receptor (ER) .As an example of how this pathway might be associated with tumourigenesis, cancer can be promoted by estrogen or estrogen agonist via the ER. Vitamin A and the RAR are critical in epithelial – mesenchymal interaction (Batourina et al., 2001) (http://www.stke.sciencemag.org)

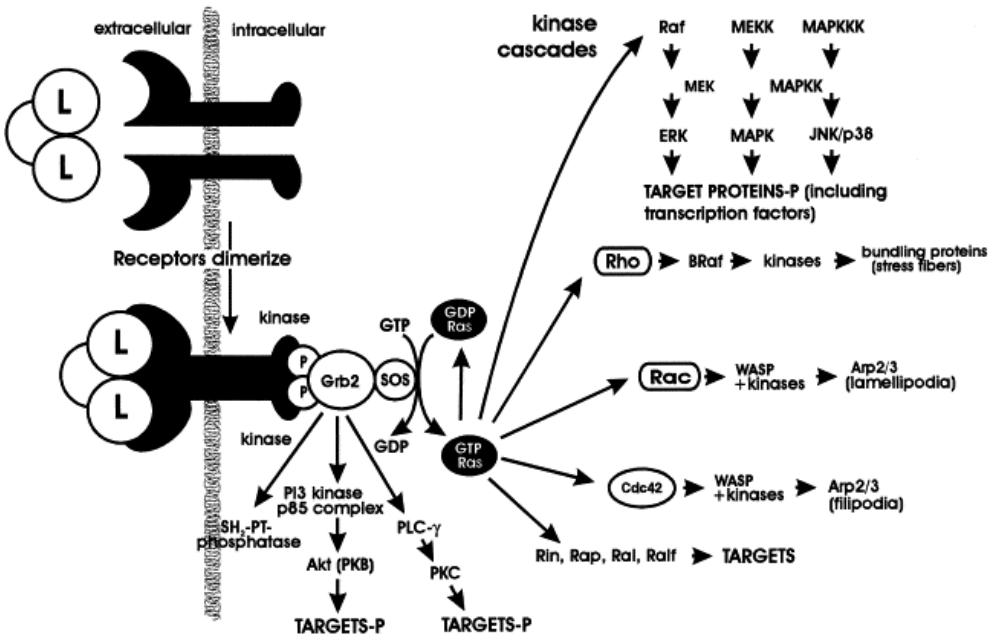

Figure4. Illustration of the receptor tyrosine kinase signal transduction pathway, and its associated MAP kinase cascades that function in response to oxidative stress. As an example of how this pathway might be associated with tumourigenesis, RAS and RAF are proto-oncogenes (Blume-Jensen and Hunter, 2001).

2.1 Switching off NK cell by the interaction with MHC molecule

In humans, NK cell display HLA class 1 specific inhibitory receptor, these also known as KIR. KIR detects shared allelic determinants of HLA class 1 molecule (HERBERMAN R.B., DJEU J.Y., KAY D.H. et al. 1979). While other display a large range of specificity for different HLA class 1 molecule (LIR1/ILT2), in normal condition NK cell express at least one receptor specific for self HLA 1 class, simultaneously expression of two or more than two self reactive receptors

is infrequent. This property of NK cell pool of a particular individual to sense the loss of even single class 1 allele on self cells.

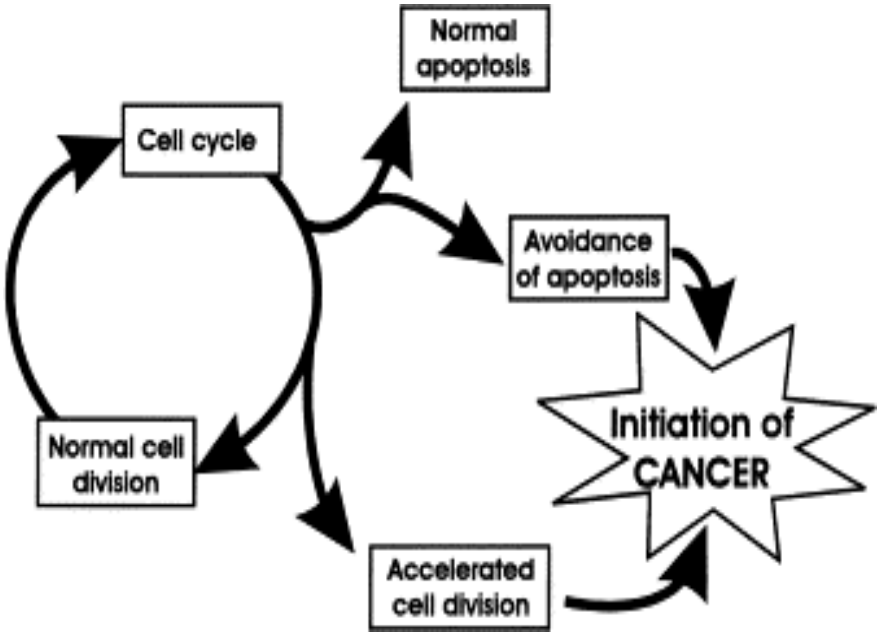

Figure5. Simplified diagram denoting the interrelationship between abnormalities in the cell cycle and initiation of cancer. The earliest beginnings of cancer most likely must include perturbation in the normal cell cycle (cf. recent review by Evan and Vousden, 2001)

Chapter3 MATERIAL AND METHODS

3.1 SOFTWARE AND TOOLS

3.1.1 CONTRA V3

The ConTra series of tools have been designed to properly display predicted TFBSs in several possible alignments aiming to help the biologist seeking to generate or support a hypothesis (Yanez et al. 2012) (Slattery. M et al. 2011). The ConTra v3 frontend has been completely re-implemented using latest web technologies to meet the required level of interactivity and user involvement. New features include a new layout, a simpler submission form, an on-screen guide and a dynamic TFBS viewer. The simplified design of the website layout facilitates user interaction and brings the main focus on the information provided. ConTra allows users of different screen sized devices to use the service without troubles (Hooghe, B. et al. 2008). Use interface form itself was simplified both visually and practically, allowing the user to have a better understanding of the required data and a clearer overview of the provided input (Broos, S et al 2011). TFBS visualization images but also a dynamic in TFBS viewer, where the user can select TFs and zoom in on the identified binding sites. ConTra uses the TRANSFAC database (update 2011.3), the JASPAR core database (update 2016), the cisBP Homo sapiens database and the Taipale motifs collection for visualization. PWM libraries that were seldom used according to web logs, such as the phyloFACTS database and a collection of homeodomain PWMs derived from a protein binding microarray.

3.1.2 NCBI BLAST

In bioinformatics, BLAST for Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

INPUT

Input sequences (in FASTA or Genbank format) and weight matrix

OUTPUT

BLAST output can be delivered in a variety of formats. These formats include HTML, plain text, and XML formatting. For NCBI's web-page, the default format for output is HTML. When performing a BLAST on NCBI, the results are given in a graphical format showing the hits found, a table showing sequence identifiers for the hits with scoring related data, as well as alignments for the sequence of interest and the hits received with corresponding BLAST scores for these. The easiest to read and most informative of these is probably the table.

3.1.3 ENSEMBL

The Ensembl project focuses on the chordates includes the genome of over 50 species including human Mouse rat and zebrafish most of the chordates are mammals and the non chordates genome c. elegans yeast and fruit fly also included and aid in more accurate phylogenetic gene trees and symbol analysis is now extended to bacterial plants, protist, and fungi. In the gene built cDNA and protein sequences from the sources such as NCBI reference sequences set a uniprot are aligned to the genome from these alignments transcript are clusters together based on

overlapping coding sequence to form ensemble gene. This is the automatic annotation pipeline for some species such as human manually curated transcript from the Vega Havana project welcome trust Sanger Institute. Ensembl provide genes for even newly sequenced low coverage genomes such as elephant dolphin cat. Other annotation includes variation mapping comparative genomic studies and functional genomics. Start at the ensembl main page we could search the gene by gene ID or name, chromosomal region and a probe set or even a disease, and then click on the homo sapiens to move to the species specific home page for the human, here we find the information about the Assembly. Now we will search for the gene by typing gene name at the top of page and click on the go button.

3.2 Methodology

For prediction of transcription factor binding site I am using Contra V3 and ensemble for extracting the 5' UTR. And to find the exact genomic position of 5' UTR in the gene here I am using NCBI blast.

3.2.1 Extracting 5' UTR from the gene by using ensemble

We could export the 5' UTR by using ensembl. On the home page of ensemble enter the gene name and getting another page. On this page there were many transcript IDs of the particular gene then I select particular transcript and click on the gene annotation now I am in the transcript tab and click on the export data. Then there were many options for FASTA sequence these are genomic c-DNA, coding sequences, peptide sequences 5'utr, exons, introns then I select 5' UTR from the options then click on the next and get 5' UTR sequence.

3.2.2 Finding exact genomic position of 5' UTR

To find the exact chromosomal location of 5' UTR in the gene I am using NCBI BLAST tool. In which, at the place of query sequence 5' UTR sequence is used and for the target sequence whole gene sequence is used. BLAST will find the exact region which is similar in both the sequences and position of query sequence in the target sequence.

3.2.3 Prediction of transcription factor binding site

INPUT

A typical ConTra v3 analysis consists of four steps. First, users have to choose whether they want to visualize or explore a gene of interest. For visualization, it is also necessary to indicate the reference species and the gene of interest. The second step lists a group of available transcripts for genes matching the search terms, from which one can be selected. For every gene, all possible RefSeq and Ensembl transcript variants are listed with a link to the genomic location in the respective genome browser. This way, genes with alternative promoters, UTRs or alternative intronic regions can be analyzed for regulatory differences. In step 3, different genomic regions of the selected transcript can be chosen (upstream, introns, 5' UTR and 3' UTR). The final step offers users an extensive choice of PWM motifs: up to 20 PWM motifs can be simultaneously taken into account for analysis.

OUTPUT

For the visualization part, results are split into alignment blocks allowing evaluation of the degree of binding site conservation.

Chapter 4 RESULTS AND DISCUSSION

4.1 Binding of TF with KIR2DL1

Killer cell immunoglobulin-like receptor 2DL1 is a protein that in humans is encoded by the KIR2DL1 gene. Killer-cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed by natural killer cells and subsets of T cells. The KIR genes are polymorphic and highly homologous and they are found in a cluster on chromosome 19q13.4 within the 1 Mb leukocyte receptor complex (LRC). The gene content of the KIR gene cluster varies among haplotypes, although several "framework" genes are found in all haplotypes (KIR3DL3, KIR3DP1, KIR2DL4, KIR3DL2. KIR2DL1 is one of the inhibitory receptor of the NK cell. Here, with the help of ConTra v3 software we shown binding of many transcription factor with KIR2DL1.some transcription factors shown binding on the KIR2DL1 gene indicates that this receptor gene is also regulate by the tumour derived transcription factor. TF which shows' binding with the gene and their location of binding in different organisms is indicated by the same color code in the TF list. ER beta and GATA -3 shows binding with KIR2DL1, so these transcription factor can regulate the gene expression of the KIR2DL1 gene and affect the activity of NK cell in response to tumour cell.

Reference organism:
Human (*Homo sapiens*)
Genomic position:
chr19:54769811-54769852

TRANCRIPTION FACTOR LIST 1 of KIR2DL1

ATF2 Homo_sapiens,M0300_1.02,M0300_1.02
ELK1 Homo_sapiens,M6207_1.02,M6207_1.02
NFKB1 Homo_sapiens,M1928_1.02,M1928_1.02
NF-kappaB TRANSFAC20113,V$NFKB_Q6,M00194

E2F1 Homo_sapiens,M4536_1.02,M4536_1.02

TBP Homo_sapiens,M4708_1.02,M4708_1.02

TP53 Homo_sapiens,M1929_1.02,M1929_1.02

Stp2 TRANSFAC20113,F$STP2_01,M01560

TCF12 Homo_sapiens,M4479_1.02,M4479_1.02

Tcf12 JASPAR_CORE_2016,MA0521.1,MA0521.1

TCF15 Homo_sapiens,M4850_1.02,M4850_1.02

ERalpha TRANSFAC20113,V$ERALPHA_01,M01801

ER-beta TRANSFAC20113,V$ERBETA_Q5,M01875

AHR Homo_sapiens,M6139_1.02,M6139_1.02

Arnt JASPAR_CORE_2016,MA0004.1,MA0004.1

FXR/RXR-alpha TRANSFAC20113,V$FXR_Q3,M00631

GATA-3 TRANSFAC20113,V$GATA3_03,M00351

Helios A TRANSFAC20113,V$HELIOSA_02,M01004

C-ets-1 TRANSFAC20113,V$CETS1_Q6,M01870

KLF4 Homo_sapiens,M6324_1.02,M6324_1.02

BINDING RESULT OF LIST 1

HUMAN ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT

CHIMP ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT

GORILLA ACAAACTGTTCAATACATTCAATTTCAAGAGGCCATTTCAAT

RHESUS ACAAACTGTTCAAAACATCCAATTTTAAGAGGCCATTTCAAT

BABOON ACAAACTGTTCAATACATCCAATTTTAAGAGGCCATTTCAAT

MARMOSET ACAAACTGTTCAATACATCCAATTTTAAGGGGCCATTTCAAT

TARSIER ---------- TGCATCCATTTTTAAGAGGCCATTGCAAT

MOUSE LEMUR CCACACTAGGCATTACATCCATTTCTCAGAGGCCATTGCAGT

TRANCRIPTION FACTOR LIST 2 of KIR2DL1

N-Myc TRANSFAC20113,V$NMYC_01,M00055

FOS Homo_sapiens,M2278_1.02,M2278_1.02

BATF::JUN JASPAR_CORE_2016,MA0462.1,MA0462.1

MET28 JASPAR_CORE_2016,MA0332.1,MA0332.1

MET28 TRANSFAC20113,F$MET28_01,M01674

c-Myc TRANSFAC20113,V$CMYC_01,M01145

c-Myc TRANSFAC20113,V$CMYC_02,M01154

ARALYDRAFT_484486 JASPAR_CORE_2016,MA1098.1,MA1098.1

p53 TRANSFAC20113,V$P53_01,M00034

p53 TRANSFAC20113,V$P53_02,M00272

p53 TRANSFAC20113,V$P53_05,M01655

p53 TRANSFAC20113,V$P53_04,M01652

P53 TRANSFAC20113,V$P53_03,M01651

TP53 Homo_sapiens,M1929_1.02,M1929_1.02

BINDING RESULT OF LIST 2

HUMAN ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT

CHIMP ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT

GORILLA ACAAACTGTTCAATACATTCAATTTCAAGAGGCCATTTCAAT

RHESUS ACAAACTGTTCAAAACATCCAATTTTAAGAGGCCATTTCAAT

BABOON ACAAACTGTTCAATACATCCAATTTTAAGAGGCCATTTCAAT

MARMOSET ACAAACTGTTCAATACATCCAATTTTAAGGGGCCATTTCAAT

TARSIER ---------- TGCATCCATTTTTAAGAGGCCATTGCAAT

MOUSE LEMUR CCACACTAGGCATTACATCCATTTCTCAGAGGCCATTGCAGT

TRANCRIPTION FACTOR LIST 3 of KIR2DL1

C-ets-1 TRANSFAC20113,V$CETS1_Q6,M01870
KLF4 Homo_sapiens,M6324_1.02,M6324_1.02
AP-1 TRANSFAC20113,V$AP1_Q4,M00188
AP-1 TRANSFAC20113,V$AP1_Q4_01,M00926
AP-1 TRANSFAC20113,V$AP1_C,M00199
AP-1 TRANSFAC20113,V$AP1_Q2_01,M00924
AP-1 TRANSFAC20113,V$AP1_01,M00517
AP-1 TRANSFAC20113,V$AP1_Q6,M00174
AP-1 TRANSFAC20113,V$AP1_Q2,M00173
AP-1 TRANSFAC20113,V$AP1FJ_Q2,M00172
NF-kappaB TRANSFAC20113,V$NFKB_Q6_01,M00774
c-Rel TRANSFAC20113,V$CREL_01,M00053
RELB Homo_sapiens,M6448_1.02,M6448_1.02
NFATC1 taipale,taipale-TTTCCAYNRTGGAAA-NFATC1,taipale-TTTCCAYNRTGGAAA-NFATC1
GLI TRANSFAC20113,V$GLI_Q2,M01037
FOXO1 Homo_sapiens,M6245_1.02,M6245_1.02
E4BP4 TRANSFAC20113,V$E4BP4_01,M00045
PURA Homo_sapiens,M6442_1.02,M6442_1.02

BINDING RESULT OF LIST 3

HUMAN	ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT
CHIMP	ACAAACTGTTCAATACATCCAATTTCAAGAGGCCATTTCAAT
GORILLA	ACAAACTGTTCAATACATTCAATTTCAAGAGGCCATTTCAA
RHESUS	ACAAACTGTTCAAAACATCCAATTTTAAGAGGCCATTTCAAT

BABOON ACAAACTGTTCAATACATCCAATTTTAAGAGGCCATTTCAAT

MARMOSET ACAAACTGTTCAATACATCCAATTTTAAGGGGCCATTTCAA

TARSIER ----------TGCATCCATTTTTAAGAGGCCATTGCAAT

MOUSE LEMUR CCACACTAGGCATTACATCCATTTCTCAGAGGCCATTGCAGT

4.2 NKG2C

NKG2-C type II integral membrane protein is a protein that in humans is encoded by the KLRC2 gene; Here NKG2C does not show any binding with the transcription factor.

Reference organism:

Human (*Homo sapiens*)

Genomic position:

chr12:10435986-10435993

TRANCRIPTION FACTOR LIST 1 of NKG2C

N-Myc TRANSFAC20113,V$NMYC_01,M00055

NMYC TRANSFAC20113,V$NMYC_02,M01808

ER-beta TRANSFAC20113,V$ERBETA_Q5,M01875

FOS Homo_sapiens,M2278_1.02,M2278_1.02

JUN Homo_sapiens,M2289_1.02,M2289_1.02

ESRRB Homo_sapiens,M6215_1.02,M6215_1.02

Rarb JASPAR_CORE_2016,MA0857.1,MA0857.1

TP53 Homo_sapiens,M1929_1.02,M1929_1.02

TCF7L1 Homo_sapiens,M5903_1.02,M5903_1.

TCF15 Homo_sapiens,M4850_1.02,M4850_1.02

TFAP2D Homo_sapiens,M6146_1.02,M6146_1.02

TFAP2A Homo_sapiens,M1838_1.02,M1838_1.02

TFAP2B Homo_sapiens,M6144_1.02,M6144_1.02

FOXC2 Homo_sapiens,M6236_1.02,M6236_1.02

TWIST1 Homo_sapiens,M6527_1.02,M6527_1.02

TWIST2 Homo_sapiens,M0177_1.02,M0177_1.02

BINDING RESULT OF LIST 1

HUMAN	AGAGTGG
TARSIER	AGAGTGG
MARMOSET	AGAGTGG
BABOON	AGAGTGG
REHSUS	AGAGTGG
ORANGUTAN	AGAGTGG
CHIMP	AGAGTGG

TRANCRIPTION FACTOR LIST 2 of NKG2C

NFKB1 Homo_sapiens,M1928_1.02,M1928_1.02

NFKB2 Homo_sapiens,M6370_1.02,M6370_1.02

RELB Homo_sapiens,M6448_1.02,M6448_1.02

NFATC1 Homo_sapiens,M6361_1.02,M6361_1.02

ELK1 Homo_sapiens,M6207_1.02,M6207_1.02

GLI1 Homo_sapiens,M6264_1.02,M6264_1.02

GLI2 Homo_sapiens,M6265_1.02,M6265_1.02

GATA-3 TRANSFAC20113,V$GATA3_03,M00351

Helios A TRANSFAC20113,V$HELIOSA_02,M01004

Helios A TRANSFAC20113,V$HELIOSA_01,M01003

(SATB1)2 TRANSFAC20113,V$SATB1_01,M01232

Blimp-1 TRANSFAC20113,V$BLIMP1_Q6,M01066

EOMES Homo_sapiens,M6211_1.02,M6211_1.02

ETS1 Homo_sapiens,M4461_1.02,M4461_1.02

KLF4 Homo_sapiens,M6324_1.02,M6324_1.02

AP-1 TRANSFAC20113,V$AP1_Q6_01,M00925

LEF1, TCF1 TRANSFAC20113,V$LEF1TCF1_Q4,M00978

ERalpha TRANSFAC20113,V$ERALPHA_01,M01801

ER-beta TRANSFAC20113,V$ERBETA_Q5,M01875

v-ErbA TRANSFAC20113,V$T3R_01,M00239

BINDING RESULT OF LIST 2

HUMAN	AGAGTGG
TARSIER	AGAGTGG
MARMOSET	AGAGTGG
BABOON	AGAGTGG
REHSUS	AGAGTGG
ORANGUTAN	AGAGTGG
CHIMP	AGAGTGG

4.3 Binding of TF with NKG2A

CD94/NKG2 is a family of C-type lectin receptors which are expressed predominantly on the surface of NK cells and a subset of $CD8^+$ T-lymphocyte. These receptors stimulate or inhibit cytotoxic activity of NK cells, therefore they are divided into activating and inhibitory receptors according to their function. CD94/NKG2 recognize nonclassical MHC glycoproteins class I (HLA-E in human and Qa-1 molecules in the mouse).In this result NKG2A receptor shows high affinity to different transcription factor which can regulate their expression on the surface of NK cell. Here NKG2A shows binding with transcription factor RARB, NFATC1, HELIOS A, ER alpha, so these transcription factors can regulate the gene expression of the NKG2A receptor.

Human (*Homo sapiens*)
Genomic position:
chr12:10443029-10443166

TRANCRIPTION FACTOR LIST 1 of NKG2A

N-Myc TRANSFAC20113,V$NMYC_01,M00055
ER-beta TRANSFAC20113,V$ERBETA_Q5,M01875
FOS Homo_sapiens,M2278_1.02,M2278_1.02
ATF2:c-Jun TRANSFAC20113,V$CREBP1CJUN_01,M00041
MET28 JASPAR_CORE_2016,MA0332.1,MA0332.1
RARB Homo_sapiens,M6445_1.02,M6445_1.02
Rb:E2F-1:DP-1 TRANSFAC20113,V$E2F1DP1RB_01,M00740
p53 TRANSFAC20113,V$P53_04,M01652
TWIST1 Homo_sapiens,M6527_1.02,M6527_1.02
FOXC2 Homo_sapiens,M6236_1.02,M6236_1.02
TFAP2D Homo_sapiens,M6146_1.02,M6146_1.02
CTCFL Homo_sapiens,M4612_1.02,M4612_1.02
TCF15 Homo_sapiens,M4850_1.02,M4850_1.02

TFAP2B Homo_sapiens,M6144_1.02,M6144_1.02

NFKB1 Homo_sapiens,M1928_1.02,M1928_1.02

RELA Homo_sapiens,M4444_1.02,M4444_1.02

RELB Homo_sapiens,M6448_1.02,M6448_1.02

NFATC1 Homo_sapiens,M6361_1.02,M6361_1.02

GL1 TRANSFAC20113,P$GL1_01,M01583

GATA3 Homo_sapiens,M4665_1.02,M4665_1.02

BINDING RESULT OF LIST 1

HUMAN TAGAAGGAATTT----------AGAATAAT----------
CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACATAAT--AGAATGCCT----------
----GATACATGCACGC-A----------CAAACACA

CHIMP TAGAAGGAATTT----------AGAATAAT----------
CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAG----------
GTGATCTTATTTATATTAAAGAAAGCATTGGACATAAT--AGAATTCCT----------
----------GATACATGCACGC-A----------CAAACACA

GORIILA TAGAAGGAATTT----------AGAATAAT----------
CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACATAAT--AGAATTCCT----------
----GATACATGCACGC-A----------CAAACCCA

ORANGTUN TAGAAG----------
GAATTCAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTTATTTATATTAAAG
AAAGCATTGGACATAAT--AGAATTCCT----------
GATACATGCACGC-A----------CGAACACA

RHESUS TAGAAGGAATTT----------AGAATAAT----------
CAGAGGTAGAAGGAATTTAAAATGATTTCATATTGCTGGGAAAATTTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGATGTAAT--AGAATTCCT----------
----GATACATGCACGC-ACACACACGCACACACGCACACACA

BABOON TAGAAGGAATTT----------AGAATAAT----------
CAGAGGTAGAAGGAATTTAAAATGATTTCATATTGCTGGGAAAATTTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACGTAAT--AGAATTCCT----------
----GATACATGCACGC-ACACACACGCACACACGCACACACA

MARMOSET ----------CA----------AGGATAAT----------
CAGAGGCAGAAGGACTTTAAAGTGATTTCATATTGCTAGGTAAATGTGTGTAATCTT
ATTTATATTAAAGAAAGCATTAAAC--AAT--AAAATTCCT----------
--GATACACACACAC-A--------CACACACACACACACA

TARSIER ----------CG----------AAAATATT----------
CAGAGCTAAGAGAAA**CTTGAA-**
TGATTTCTTACTATTAAGCATATTTACATGATCTTATTTACATTAAGAGAATTATTG
GACATAAC----AATTTCT----------TATGCGTACACAC-A----------
--------CA

BUSHBABY ----------CA----------AGAGTATT----------
CAGAGGTTAGGAGGACTCTACATAGTTCTCTTTTGCTAGGTATAGCTGGATGATCTT
ATTTACCTTAAAGAAAATATTGGATGTAAC--AGAATTTCT----------
----GCTACACACACAT-A--------TATACACACAT-CATA

MOUSE LEMUR ----------CA----------AGAATATT----------
CAGAGATTAGGCAGACTCCAAATGATTTCCTATTACTAGGTATATCTGGGTGATCTT
ATCTACGTCGAAGAAAGTATTGGACATAAC----AATTTCT----------
--GACATACACATAC-A--------CACATACACATACACG

TREESHREW ----------TA----------AAAATATC----------
TAGAGGTGGAAGGAATTTAAA-TATTTTCCTCTCAGTTAGGATATTTTTGTGATCAT---

TACAGTAAAAAAGGTATTGG----CAT--GAAATTTCT----------
GATGTAT----------

RAT ----------AGAATGCT----------
TAGAGAAAGGAAGAGTTC-------CTTGTTTCTGGGTGCATTTGTATGGTGTTCTTTACA-
TATAGATACCATTGAGGATAAA--
CATTTTTCTATCAGTAAAATAGTGGAATGCTGAAATAGTTCATTAAAAGACACTT-----
----------CCACATTCATGTACA

MOUSE ----------AAAATGTT----------
--CAGAGATAGGACAAAATC----------
CTTGTTGCTGGGTATATTTGTATGATTCTTTTTACA-
TACAGATACCATTATGGGTAAT--
TCTTTTTCTATCAGTAAAATAGTGGAATGCCAAAATACTTCATTAAGAGATACAT-----
----------TTATATTCATGCACA

PIKA ----------C----------GATGTATT----------
AAGAGGCAGGAAGGGTTCATA-TGATTTCCTACTTACAGGTATAT----------

RABBIT ----------CA----------AAAATATT----------
-AAGAGGCAGGAGAGATTTAAA-TGATTTCCTATTGGTAGGT----------

DOLPHIN ----------AGATTATT----------
----------AAA-TGATTTCCTATTGCTAGG**TATACTTGTGTGACCTT**---
CACTTTAAAGAAGGTATTGCAC---AG--AGAATTTCT----------
GGTGTAT----------

HORSE ----------AGAATATT----------
CAAACATAGGAGAAATTTAAA-TGATATCTTATTGCTAGGTATGCTTGTGTAATCTT--
-TACATTAAGGAAAGTATTGGATGTGAC----AATTTCT----------
GGTGTAT--ATAC-A---------CTTAATGTGTACTGA

DOG ----------AGAATATT----------
-CAAA----------GGTGGTCTATTGCTAGGTTTACTTGTATAATCTT---
TACATTAAAGAAAGTATTGGATAAAAC--AGAATATCT----------
GGTGTGT--GTACTG---------GTAAATGTATGCTCA

MEGABAT ----------AAAATATT----------
CAGAAGTTGGAGAAATTTCAA-TGCTTTCCTAATGCTAGGTATATGTGTATGATCTT--
-CATAGTAAAGAAAGTATTGCACAAAAG--AGAATTTCT----------
GGTGTAT--ATAT-A---------CTTAACGTATACTCA

SHREW ----------ACTAATCGATGGTGTGGCAAATGTATT----------CAGAGGTGAAAGAAAC--AAA-TTAAGTGTCGTAGTTA-GTATGC-
TATGAGATCTT---CACATAAAAGAAAGTATTGCACATAGC--ATATTCATT----------GGCATCT--TTCT-G---------CAGCAGTAAGACTCT

ELEPHANT -------GGACA----------AGAATATTCAGATTAGA-
--------AGAATGACTG---
TAGGTATATTTAGAAGGACTTCCTACTGCTAGGTATACTTGTGTGA---
TCTTTACATTGAAGAAAATATTATTCGTAACTGGGAATTTCT----------
-------GTTATGT----------TATTAATAAATT

ROCK HYRAX -------GAACA----------AGAATATTCAGA---------
-------AGTG----------
AGGTAAATTTAGAATGCCTCCCTACTTCTAGGTATACTTGTGTGATCTTCTTTAAACT
GAATAAAATATTATCCATAACTGAGAATTTCT----------
GTTATGT----------CATTAATAAATT

TENREC -------GCACA----------
ACATTATTTACAGGTGAAGCAAATCTAGAATGATTA----------

COW ----------
-GAGGTGGGAGAAGTTTAAA-TGATTTCCTATTTCTAGGTATACTTGTGTGATCTT---

CACCCTAAAGAAGGTATTTCAT---AC--AGAATTTCT----------
AGTGTAT--ATCT-A---------CTTAAGCTATA----

TRANCRIPTION FACTOR LIST 2 of NKG2A

Helios A TRANSFAC20113,V$HELIOSA_02,M01004

Helios A TRANSFAC20113,V$HELIOSA_01,M01003

Blimp-1 TRANSFAC20113,V$BLIMP1_Q6,M01066

(Prop-1)2 TRANSFAC20113,V$PROP1_01,M01294

EOMES Homo_sapiens,M6211_1.02,M6211_1.02

ETS1 Homo_sapiens,M4461_1.02,M4461_1.02

KLF4 Homo_sapiens,M6324_1.02,M6324_1.02

AP-1 TRANSFAC20113,V$AP1_01,M00517

AP-1 TRANSFAC20113,V$AP1_Q6,M00174

TCF12 Homo_sapiens,M4479_1.02,M4479_1.02

TCF15 Homo_sapiens,M4850_1.02,M4850_1.02

ERalpha TRANSFAC20113,V$ERALPHA_01,M01801

ER-beta TRANSFAC20113,V$ERBETA_Q5,M01875

AHR Homo_sapiens,M6139_1.02,M6139_1.02

ARNT Homo_sapiens,M6151_1.02,M6151_1.02

RARA Homo_sapiens,M6443_1.02,M6443_1.02

RXRA Homo_sapiens,M4511_1.02,M4511_1.02

ATF2 Homo_sapiens,M0300_1.02,M0300_1.02

NF-kappaB TRANSFAC20113,V$NFKB_Q6_01,M00774

BINDING RESULT OF LIST 3

HUMAN TAGAAGGAATTT----------AGAATAAT----------

CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACATAAT--AGAATGCCT----------
----GATACATGCACGC-A----------CAAACACA

CHIMP TAGAAGGAATTT----------AGAATAAT----------
-----CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAG----------
GTGATCTTATTTATATTAAAGAAAGCATTGGACATAAT--AGAATTCCT----------
----------GATACATGCACGC-A----------CAAACACA

GORIILA TAGAAGGAATTT----------AGAATAAT----------

CAGAGGTAGAAAGAATTTAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACATAAT--AGAATTCCT----------
----GATACATGCACGC-A----------CAAACCCA

ORANGTUN TAGAAG----------

GAATTCAAAATGATTTCATATTGCTAGGTAAATCTGTGTGATCTTATTTATATTAAAG
AAAGCATTGGACATAAT--AGAATTCCT----------
GATACATGCACGC-A----------CGAACACA

RHESUS TAGAAGGAATTT----------AGAATAAT----------

CAGAGGTAGAAGGAATTTAAAATGATTTCATATTGCTGGGAAAATTTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGATGTAAT--AGAATTCCT----------
----GATACATGCACGC-ACACACACGCACACACGCACACACA

BABOON TAGAAGGAATTT----------AGAATAAT----------

CAGAGGTAGAAGGAATTTAAAATGATTTCATATTGCTGGGAAAATTTGTGTGATCTT
ATTTATATTAAAGAAAGCATTGGACGTAAT--AGAATTCCT----------
----GATACATGCACGC-ACACACACGCACACACGCACACACA

MARMOSET ----------CA----------AGGATAAT----------
--
CAGAGGCAGAAGGACTTTAAAGTGATTTCATATTGCTAGGTAAATGTGTGTAATCTT
ATTTATATTAAAGAAAGCATTAAAC--AAT--AAAATTCCT----------
--GATACACACACAC-A--------CACACACACACACACA

TARSIER ----------CG----------AAAATATT----------
CAGAGCTAAGAGAAACTTGAA-
TGATTTCTTACTATTAAGCATATTTACATGATCTTATTTACATTAAGAGAATTATTGG
ACATAAC----AATTTCT----------TATGCGTACACAC-A----------
------CA

BUSHBABY ----------CA----------AGAGTATT----------
CAGAGGTTAGGAGGACTCTACATAGTTCTCTTTTGCTAGGTATAGCTGGATGATCTT
ATTTACCTTAAAGAAAATATTGGATGTAAC--AGAATTTCT----------
----GCTACACACACAT-A--------TATACACACAT-CATA

MOUSE LEMUR ----------CA----------AGAATATT----------
CAGAGATTAGGCAGACTCCAAATGATTTCCTATTACTAGGTATATCTGGGTGATCTT
ATCTACGTCGAAGAAAGTATTGGACATAAC----AATTTCT----------
--GACATACACATAC-A--------CACATACACATACACG

TREESHREW ----------TA----------AAAATATC----------
TAGAGGTGGAAGGAATTTAAA-TATTTTCCTCTCAGTTAGGATATTTTTGTGATCAT---
TACAGTAAAAAAGGTATTGG----CAT--GAAATTTCT----------
GATGTAT----------

RAT ----------AGAATGCT----------
TAGAGAAAGGAAGAGTTC----------
CTTGTTTCTGGGTGCATTTGTATGGTGTTCTTTACA-

TATAGATACCATTGAGGATAAA--
CATTTTTCTATCAGTAAAATAGTGGAATGCTGAAATAGTTCATTAAAAGACACTT-----
----------CCACATTCATGTACA

MOUSE ----------AAAATGTT----------
CAGAGATAGGACAAAATC----------
CTTGTTGCTGGGTATATTTGTATGATTCTTTTTACA-
TACAGATACCATTATGGGTAAT--
TCTTTTTCTATCAGTAAAATAGTGGAATGCCAAAATACTTCATTAAGAGATACAT-----
----------TTATATTCATGCACA

PIKA ----------C----------GATGTATT----------
AAGAGGCAGGAAGGGTTCATA-TGATTTCCTACTTACAGGTATAT----------

RABBIT ----------CA----------AAAATATT----------
AAGAGGCAGGAGAGATTTAAA-TGATTTCCTATTGGTAGGT----------

DOLPHIN ----------AGATTATT----------
----------AAA-TGATTTCCTATTGCTAGGTATACTTGTGTGACCTT---
CACTTTAAAGAAGGTATTGCAC---AG--AGAATTTCT----------
GGTGTAT----------

HORSE ----------AGAATATT----------
CAAACATAGGAGAAATTTAAA-TGATATCTTATTGCTAGGTATGCTTGTGTAATCTT--
-TACATTAAGGAAAGTATTGGATGTGAC----AATTTCT----------
GGTGTAT--ATAC-A---------CTTAATGTGTACTGA

DOG ----------AGAATATT----------
CAAA----------GGTGGTCTATTGCTAGGTTTACTTGTATAATCTT---
TACATTAAAGAAAGTATTGGATAAAAC--AGAATATCT----------
GGTGTGT--GTACTG---------GTAAATGTATGCTCA

MEGABAT ----------AAAATATT----------
----CAGAAGTTGGAGAAATTTCAA-
TGCTTTCCTAATGCTAGGTATATGTGTATGATCTT---
CATAGTAAAGAAAGTATTGCACAAAAG--AGAATTTCT----------
GGTGTAT--ATAT-A---------CTTAACGTATACTCA

SHREW ----------ACTAATCGATGGTGTGGCAAATGTATT--
----------CAGAGGTGAAAGAAAC--AAA-TTAAGTGTCGTAGTTA-GTATGC-
TATGAGATCTT---CACATAAAAGAAAGTATTGCACATAGC--ATATTCATT----------
----------GGCATCT--TTCT-G---------CAGCAGTAAGACTCT

ELEPHANT -------GGACA----------
AGAATATTCAGATTAGA---------AGAATGACTG-----
TAGGTATATTTAGAAGGACTTCCTACTGCTAGGTATACTTGTGTGA---
TCTTTACATTGAAGAAAATATTATTCGTAACTGGGAATTTCT----------
-------GTTATGT----------TATTAATAAATT

ROCK HYRAX -------GAACA----------AGAATATTCAGA---
----------AGTG----------
AGGTAAATTTAGAATGCCTCCCTACTTCTAGGTATACTTGTGTGATCTTCTTTAAACT
GAATAAAATATTATCCATAACTGAGAATTTCT----------
GTTATGT----------CATTAATAAATT

TENREC -------GCACA----------
ACATTATTTACAGGTGAAGCAAATCTAGAATGATTA----------

COW ----------
GAGGTGGGAGAAGTTTAAA-TGATTTCCTATTTCTAGGTATACTTGTGTGATCTT---
CACCCTAAAGAAGGTATTTCAT---AC--AGAATTTCT----------
AGTGTAT--ATCT-A---------CTTAAGCTATA----

SUMMRY TABLE OF RESULT

GENE NAME	TRANSCRIPTION FACTOR	SEQUENCE	ORGANISM
KIR2DL1	ER-beta	AGGGGCCATTTCAAT	MARMOSET
	GATA-3	TTACATCCAT	MOUSE LEMUR
	MET 28	ACATTCAATTTC	GORILLA
NKG2A	RARB	TATACTTGTGTGACCTT	DOLPHIN
	NFATC1	CTTGAATGATTTCTT	TARSIER
		TATGATTCTTTTT	MOUSE
	Helios A	GATTTCCTATTAC	MOUSE LEMUR
		TATTTTCCTCTCAG	TREE SHREW
		GAAAGGAAGAG	RAT
		TGATTTCCTATT	RABBIT
		TGATTTCCTACT	PIKA
		TGATTTCCTATT	DOLPHIN
		GCTTTCCTAATGCT	MEGA BAT
		TGATTTCCTATTTCT	COW
	HELIOS B	GAGGTGGGAGAA	COW
	ER ALPHA	GAAAC--AAA- TTAAGTGTCGTAGTTA	SHREW

4.4 DETAILS OF BINDING TRANCRIPTION FACTOR

4.4.1 ER-β

ER-β is a member of the family of estrogen receptors and the superfamily of nuclear receptor transcription factors. The gene product contains an N-terminal DNA binding domain and C-terminal ligand binding domain and is localized to the nucleus, cytoplasm, and mitochondria. Upon binding to 17-β-estradiol, estriol or related ligands, the encoded protein forms homo-dimers or hetero-dimers with estrogen receptor α that interact with specific DNA sequences to activate transcription. Some isoforms dominantly inhibit the activity of other estrogen receptor family members. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of some of these variants has not been fully characterized. ER-β may have anti-proliferative effects and therefore oppose the actions of ER-α in reproductive tissue. ER-β may also have an important role in adaptive function of the lung during pregnancy.

ER-β is a potent tumor suppressor and plays a crucial role in many cancer types such as prostate cancer.

4.4.2 HELIOS

Helios is a member of the Ikaros family of TFs. The role of these TFs has been studied mainly in regulatory T cells (Getnet et al., 2010) and lymphoid malignancies (Rebollo and Schmitt, 2003). It has been shown that Helios can be induced during T cell activation and proliferation (Akimova etal., 2011). Using the mice model Noe, Narni-Mancinellietal (2012)found that the absence of NKp46 expression made nk cell hyper active. The study of the transcriptome of WT mice revealed the increased expression of the TF Helios in the mature CD11b+ NK cells subset expressing NKp46. In Noe mice, Helios transcripts were twice as abundant in the cd11b+ NK

cells as compared to the same subset in WT mice. Silencing of Helios in NK cells isolated from Noe mice restored the irreactivity to the level observed for WT NK cells. The authors suggest that Helios down regulation is involved in the regulation of NK cells reactivity via Nkp46 (NarniMancinelli., 2012).

4.4.3 GATA-3

GATA - 3 is a transcription factor that play important role in T cell development and t cell differentiation. Even though the number of NK cell was reported to be normal in the spleen of GATA 3 deficient mice. NK cells in these mice were actually immature suggesting a role for GATA 3 in NK maturation but not in NK cell specification. NK cell cytotoxicity was not affected in the absence of GATA-3, however, GATA-3 deficient NK cells produced less IFN -γ then wild-type mice. This study also showed that GATA-3 was important for NK cell response *Listeria monocytogenes* infection. Moreover GATA-3 was demonstrated to be involved in the regulation of NK cell homeostasis as the number of NK cell was drastically reduced in the liver of GATA 3-/- mice. Finally Vosshenrich et al. (2006) reported that although GATA 3 was dispensable for the development of NK cells in the BM. it was indispensable for the generation of CD 127+ thymic NK cells.

4.4.4 Nfatc1

The product of this gene is a component of the nuclear factor of activated T cells DNA-binding transcription complex. This complex consists of at least two components: a preexisting cytosolic component that translocates to the nucleus upon T cell receptor (TCR) stimulation, and an inducible nuclear component. Proteins belonging to this family of transcription factors play a central role in inducible gene transcription during immune response. The product of this gene is an inducible nuclear component. It functions as a major molecular target for the immunosuppressive drugs such as ciclosporin. Five transcript variants encoding distinct isoforms have been identified for this gene. Different isoforms of this protein may regulate inducible expression of different cytokine genes.

4.4.5 Estrogen Receptor Alpha

The estrogen receptor alpha (ER-α), officially named *estrogen receptor 1* (ESR1), is a ligand-dependent nuclear hormone receptor transcription factor. The approximately 140-kB, 8-exon human *ESR1* gene is located at 6q25.1. The 5′ region of the gene contains multiple promoters responsible for tissue-specific gene expression. The encoded 595-amino-acid (66.2-kDa) protein has the domain structure typical of most nuclear hormone receptors, including amino-terminal transcription-regulating domain, a central DNA-binding domain, and a carboxy-terminal hormone-binding domain.

When ER-α is bound by its principal ligand, estradiol, the receptor-steroid complex converts to a form that binds with high affinity to nuclear components, allowing it to activate or represses the expression of numerous genes involved in a wide variety developmental, organizational, and other functions. In terms of female sex differentiation, the fundamental role of ER-α appears to be to maintain the female phenotype of the endocrine somatic cells of the ovary by inhibiting male-type (Leydig cell–like) development of interstitial (sex steroid–producing) cells. In conjunction with Wnt4, ER-α can be considered a key protector of the integrity of female sex differentiation.

4.4.6 RARB

This gene encodes retinoic acid receptor beta, a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators. This receptor localizes to the cytoplasm and to subnuclear compartments. It binds retinoic acid, the biologically active form of vitamin A which mediates cellular signalling in embryonic morphogenesis, cell growth and differentiation. It is thought that this protein limits growth of many cell types by regulating gene expression. The gene was first identified in a hepatocellular carcinoma where it flanks a hepatitis B virus integration site. The gene expresses at least two transcript variants; one additional transcript has been described, but its full length nature has not been determined

4.4.7 MET28

MET 28 TF that participate in MET pathway plays an important role in the development of cancer through:

- activation of key oncogenic pathways (RAS, PI3K, STAT3, beta-catenin);
- angiogenesis (sprouting of new blood vessels from pre-existing ones to supply a tumor with nutrients);
- Scatter (cells dissociation due to metalloprotease production), which often leads to metastasis.

Coordinated down-regulation of both MET and its downstream effector extracellular signal-regulated kinase 2 (ERK2) by miR-199a may be effective in inhibiting not only cell proliferation but also motility and invasive capabilities of tumor cells.

MET amplification has emerged as a potential biomarker of the clear cell tumor subtype.

The amplification of the cell surface receptor MET often drives resistance to anti-EGFR therapies in colorectal cancer.

Chapter 5 CONCLUSION

The transcription factor derived from tumour cell are bind on the 5' UTR of NK cell receptors genes and adversely affected their transcription rate. These tumour derived transcription factor down regulate or up regulate the signaling of the NK cell by binding on the genes that codes for the different receptor of the NK cell. The results of my experiment show that many tumour cell-derived TF show binding on the NK cell receptors gene so these transcription factors modulate the expression of inhibitory receptors on the surface of NK cell leads to tumour cell escapes from the action of NK cell. TF like ER beta, GATA 3, and MET 28 show binding with KIR2DL1 receptor gene and RARB, NFATC1, Helios A, Helios B, ER alpha show binding with NKG2A receptor gene and modulate their normal regulation by affecting their signaling pathways. These tumours derived TF up-regulate and down-regulate the signaling of NK cell and abnormalities in signaling pathway leads to progression of tumour cells. so these transcription factors can regulate the gene expression of the KIR2DL1 and NKG2A gene and affect the activity of NK cell in response to the tumour cell.

Chapter 6 References

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