BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

Course Code : 18BMS59C16

Unit-V

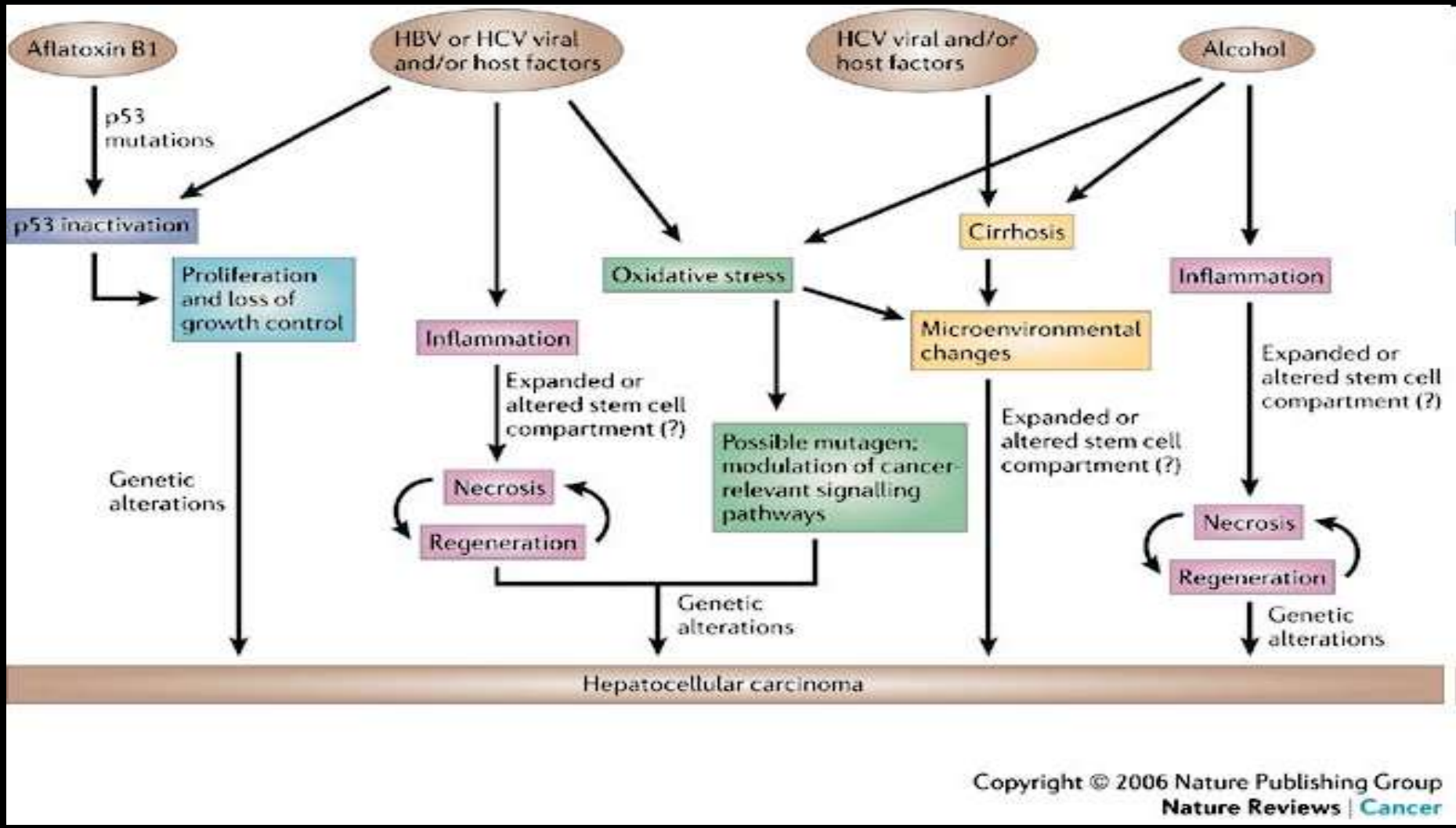
TOPIC: Strategies of Anticancer Drug Therapy

Dr. G.MATHAN
Professor
Department of Biomedical Science

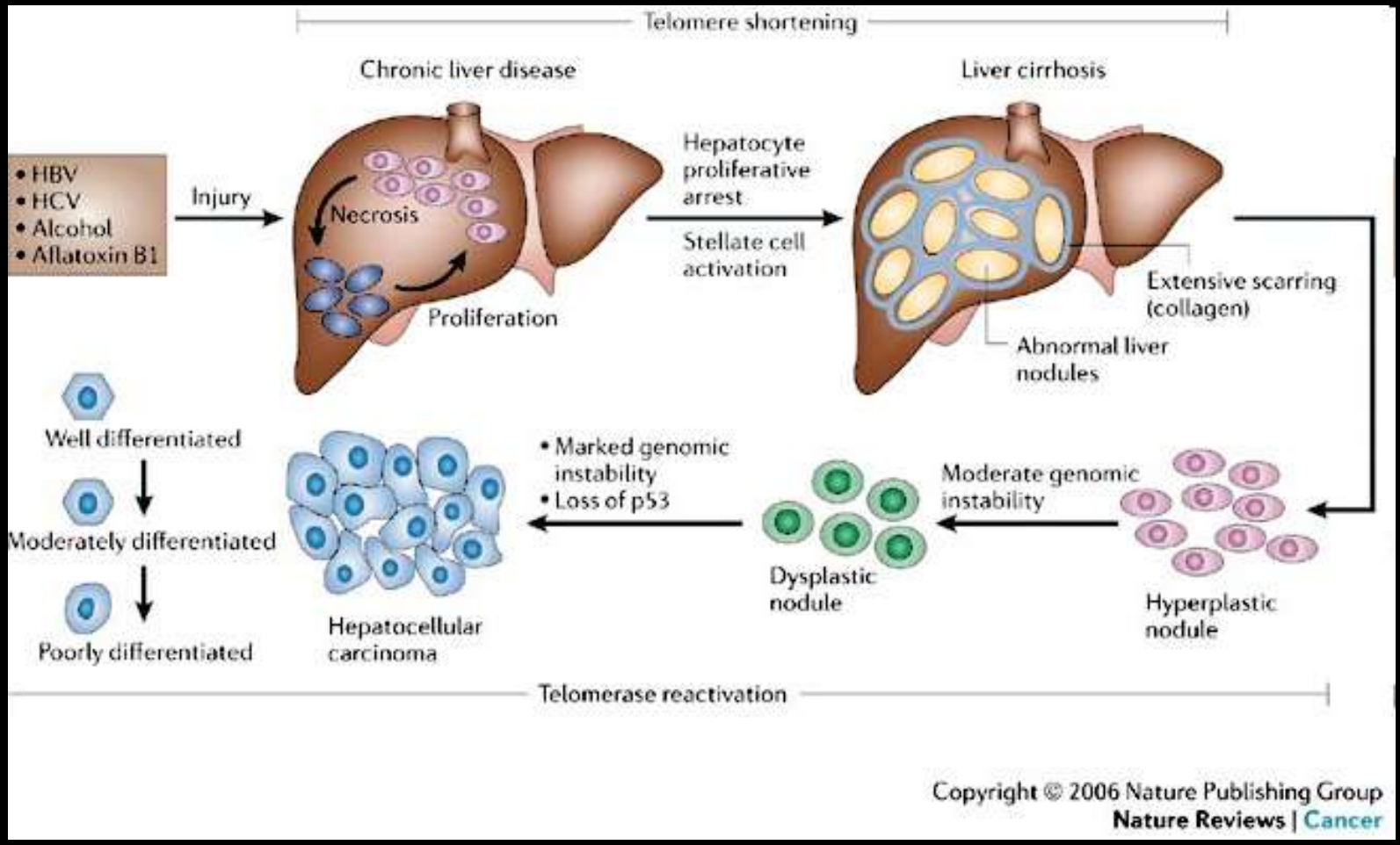
TRANSCRIPTOMICS AND NOVEL TREATMENT MODALITIES FOR HCC: A TRANSGENIC MOUSE MODEL APPROACH

Introduction

- Primary liver cancer - HCC is the most common type, accounting for 70%-85% of cases
- Peak in East-Asia and sub-Saharan Africa
- Third most frequent cause of cancer death in men and the sixth in women.
- Most of the patients currently identified in clinics are often at advanced stage of disease .
- All therapy regimens have provided limited success.



➢**Variations in the prevalence- etiological factors mirror the geographical distribution of the incidence of HCC.**



Nature Reviews | Cancer

ANIMAL MODELS

- **Carcinogen Induced Mouse models of HCC**
- **Implantation models of HCC**
- **Genetically Engineered Mouse (GEM) Models for HCC**
- **Viral Hepatocarcinogenesis-HBV and HCV associated mouse models of HCC**

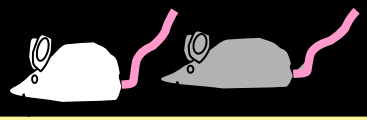
Transgenic mouse models for HCC

Transgene	Promoter	Mouse strain	Percentage HCCs	Reference
TGF-α	MT	CD1	50% in males > 12 months	1.Jhappan C, *et.al.,* 1990. 2. Lee GH, *et.al.,* 1992
c-myc	Alb	C57BL/6 × CBA/J 65	65% in males at 20 months	Santoni-Rugiu E, *et.al.,* 1996
c-myc/TGF-α	Alb, MT	C57BL/6 × CBA/J × CD1	100% in males at 8 months	Murakami *et.al.,* 1993 Santoni-Rugiu E, *et.al.,* 1996
SV40 T-Ag	AT III	C57BL/6 × DBA2	100% at 8 months	Dubois N, *et.al.,* 1991
E2F-1	Alb	C57BL/6 × CBA/J	33%-60% at 12 months	1. Conner EA, *et.al.,* 2000 2. Calvisi DF, *et.al.,* 2005
c-myc/E2F-1	Alb	C57BL/6 × CBA/J	100% at 9 months	Calvisi DF, *et.al.,* 2005

❖Alb, albumin; AT III, Antithrombin III; MT, Metallothionein

Comparative Genomic Analysis
More Humanized Mouse Model

Human HCCs



Mouse HCCs

Selection of orthologous genes

Standardization and integration of data sets
& relative expression of both species analysis

Identify best-fit mouse models for each subclass of human HCC

Identify conserved gene expression patterns in mouse and human HCC

Identify potential therapeutic targets in human HCC

Test hypothesis

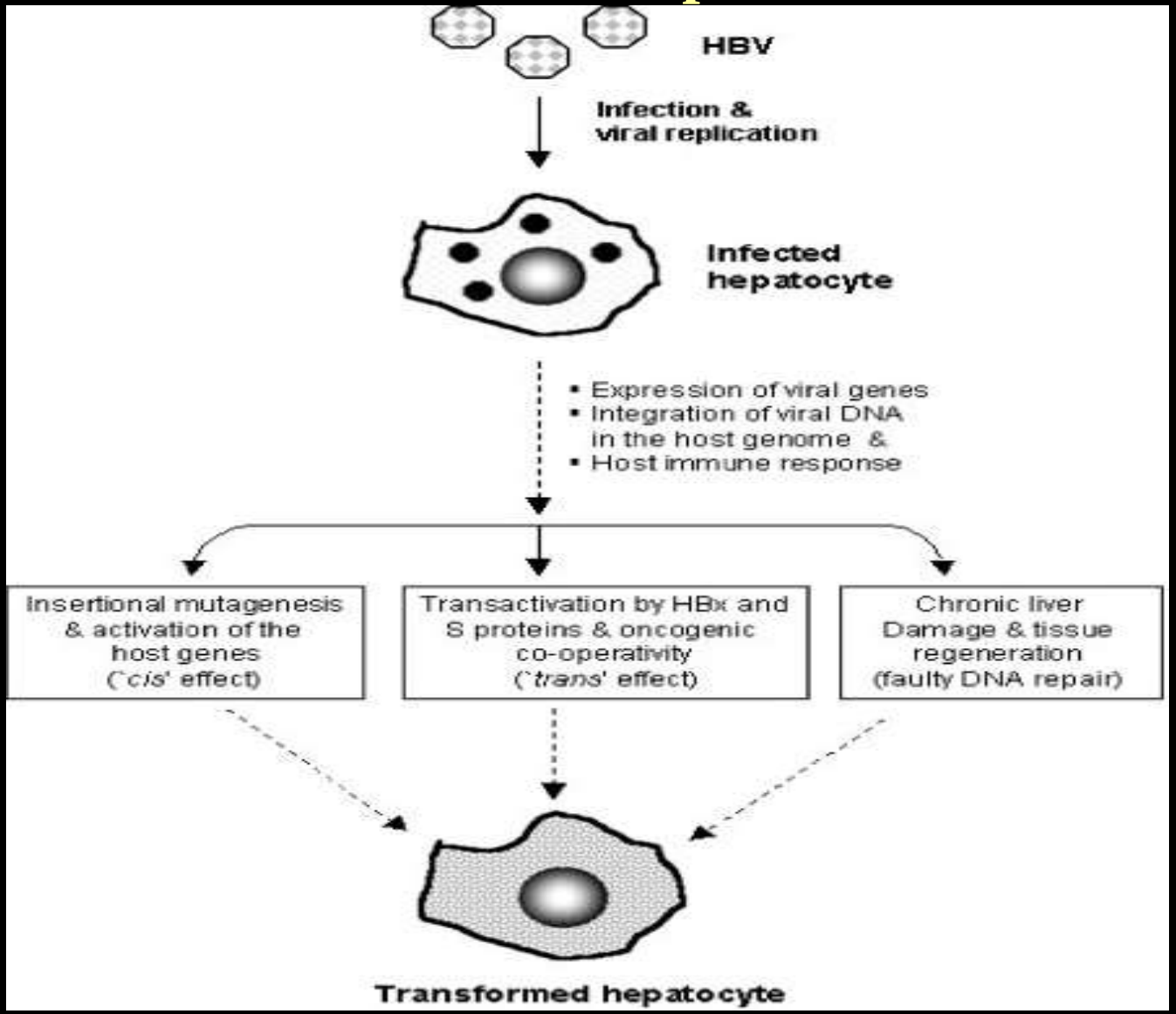
Build hypothesis

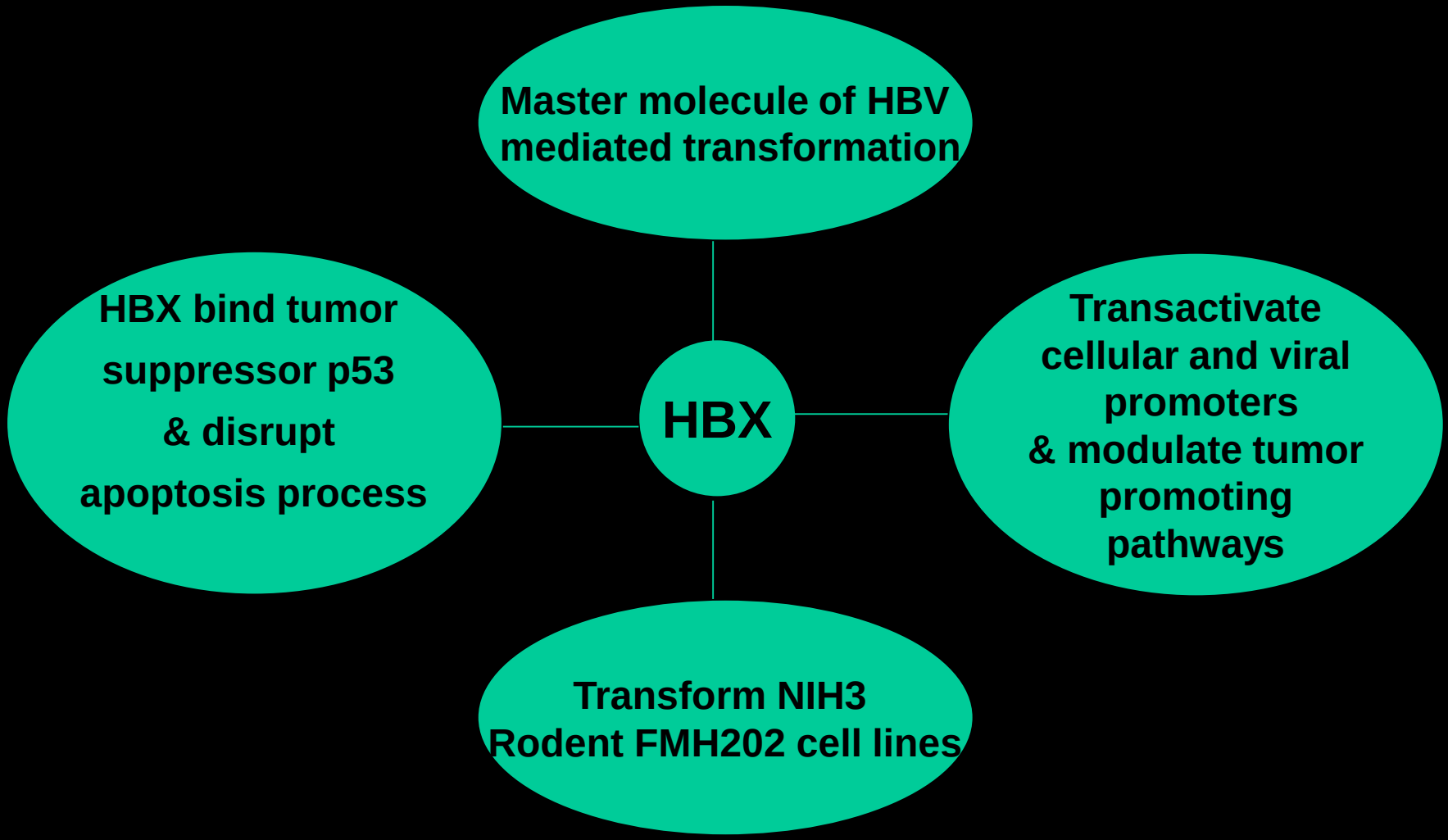
Validation and preclinical trial of targets

Viral Hepatocarcinogenesis

- More than 80% of HCC in human -HBV or HCV or both
- WHO- 400 million people-Chronic HBV-2000
- Infected person may take 20 years to develop HCC
- Undergo multiple steps of genetic alteration
- Human tropism virus
- WHV and GSHV- *in vivo* studies
- Human hepatocyte transplanted model
- Transgenic mice expressing HBV proteins represent the best model

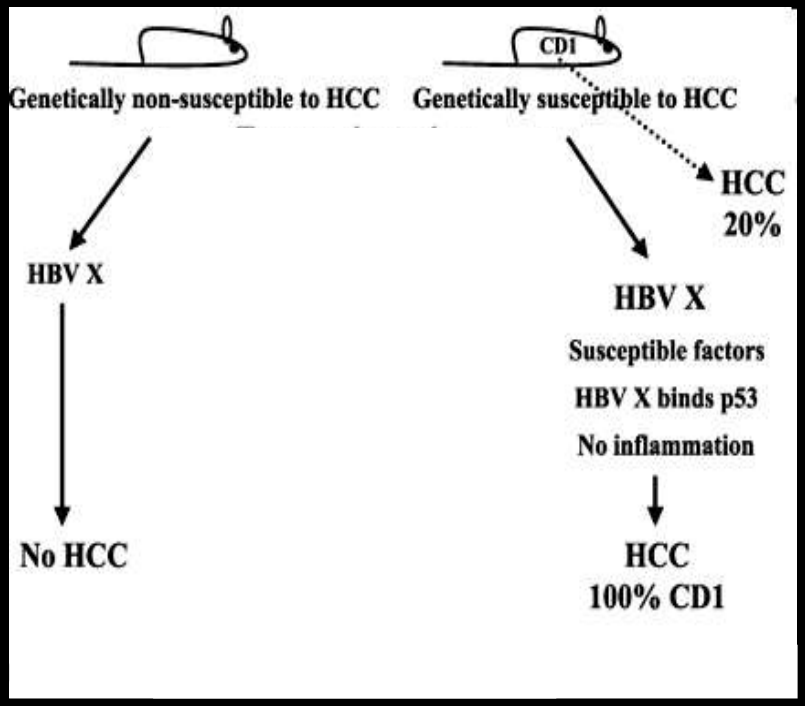
Possible mechanisms for the HBV-associated HCC development




Master molecule of HBV mediated transformation
HBX bind tumor suppressor p53 & disrupt apoptosis process
HBX
Transactivate cellular and viral promoters & modulate tumor promoting pathways
Transform NIH3 Rodent FMH202 cell lines

HBX Transgenic Mouse

CD1 background mouse model shows high spontaneous rate of HCC (Homburger et.al., 1975)



- X gene was introduced under its natural promoter.
- HBX expression ⟶ neoplastic lesions

Disadvantage:

Male animals died 11-15 months

Female mice between 17-21 months

(kim, 1991 and Koike *et.al.*, 1994)

- X transgenic mice not sufficient to study oncogenic potential of HBX and others.
- HBX-Co factor in the process of hepatopathogenesis
- Other genetic and epigenetic events may be necessary for the onset of HCC

HBV-related transgenic mouse models of HCC

Chisari FV, et.al., 1985 and Babinet C,et.al., 1985

Transgene	expressed Promoters	used Mouse strain	Pathology	Reference
Large and major S	Alb-HBV	C57BL/6 x SJL	HCC in the second half of animals' life span	Chisari FV et.al., 1989
HBx	Xp	CD1	,,	Kim CM et.al.,1991
HBx and c-myc in separate lines	Xp for HBx & WHV for c-myc	C57BL/6 x SJL/J	,,	Terradillos O,et.al., 1997
HBx	Xp	C57BL/6 x DBA	,,	Yu DY et.al., 1999

❖Alb, albumin; Cp, core gene promoter; S, surface antigen; WHV, woodchuck hepatitis virus; Xp, X gene promoter.

Accelerated HCC - HBX Tg mouse & WHV/c-myc Tg mice hybrid offspring's

But still not fast as the pathogenic studies demanded

Bicistronic DNA construct, X-myc

X15-myc
(58-150aa of X protein)-Exon 2and 3

→ **Minimal transactivation domain of HBX**
(Kumar et.al., 1996)

↓ Murine c-myc protein

Reasons for choosing myc gene

- Selective amplification of c-myc gene found in HBV related HCC cases **(Peng *et.al.*, 1993)**
- The activation of N-myc and c-myc gene is frequently observed after integration of viral **DNA (Moroy *et.al* 1996 and Fourel *et.al*., 1990)**

X15myc-Transgenic construct

Regulatory elements in HBV genome

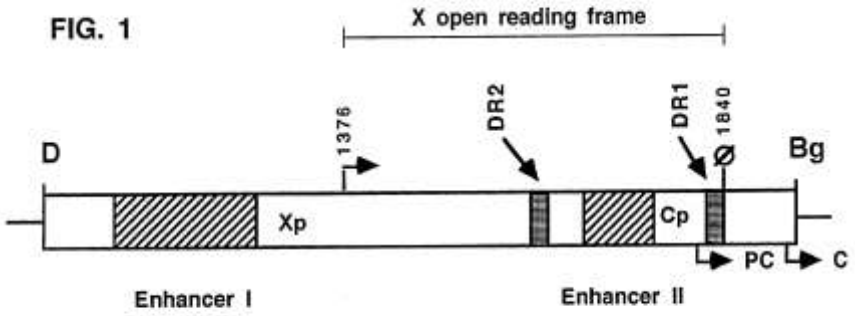
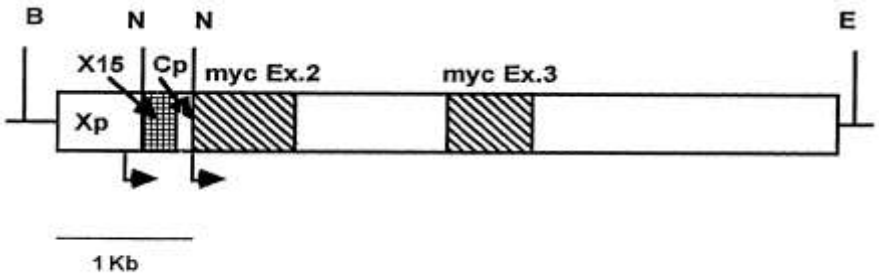


FIG. 2 X15-myc bicistronic construct



X15 region positioned to 5' to the murine c-myc gene, and is operatively linked to under the regulatory control of its natural promoter and enhancer I element.

C-myc gene is operatively linked to under the regulatory control of core promoter and enhancer II elements

This compact present construct facilitate the the core promoter and and enhancer II regions are embodied in the X gene sequence

5.7Kb EcoR I and Bam HI fragment

D-DraI, Bg-BgI II, Xp-Natural X promoter, Cp-Core promoter, B-BamhH I, N-Nco I, E-EcoR I,

X 15- myc Transgenic Mouse Model for HCC

X15- myc Transgenic Mouse

- Recombinant bicistronic construct (Singh *et al*, 2003) (Kumar *et al*, 2001) US patent no: 6274788 B1.

- X-15 myc transgenic mice appeared to be an ideal model to study the disease process and also screening drugs.

Normal Liver X15- myc Tg Liver

Transgene	expressed Promoters	used Mouse strain	Pathology	Reference
HBx and c-myc	Xp and Cp	C57BL/6 x SJL	HCC in the first half of animals' life span (3 to 5 months)	1. Singh M,et.al., 1998 2. Kumar V,et.al., 2001(us patent)

X15-myc transgenic mouse model

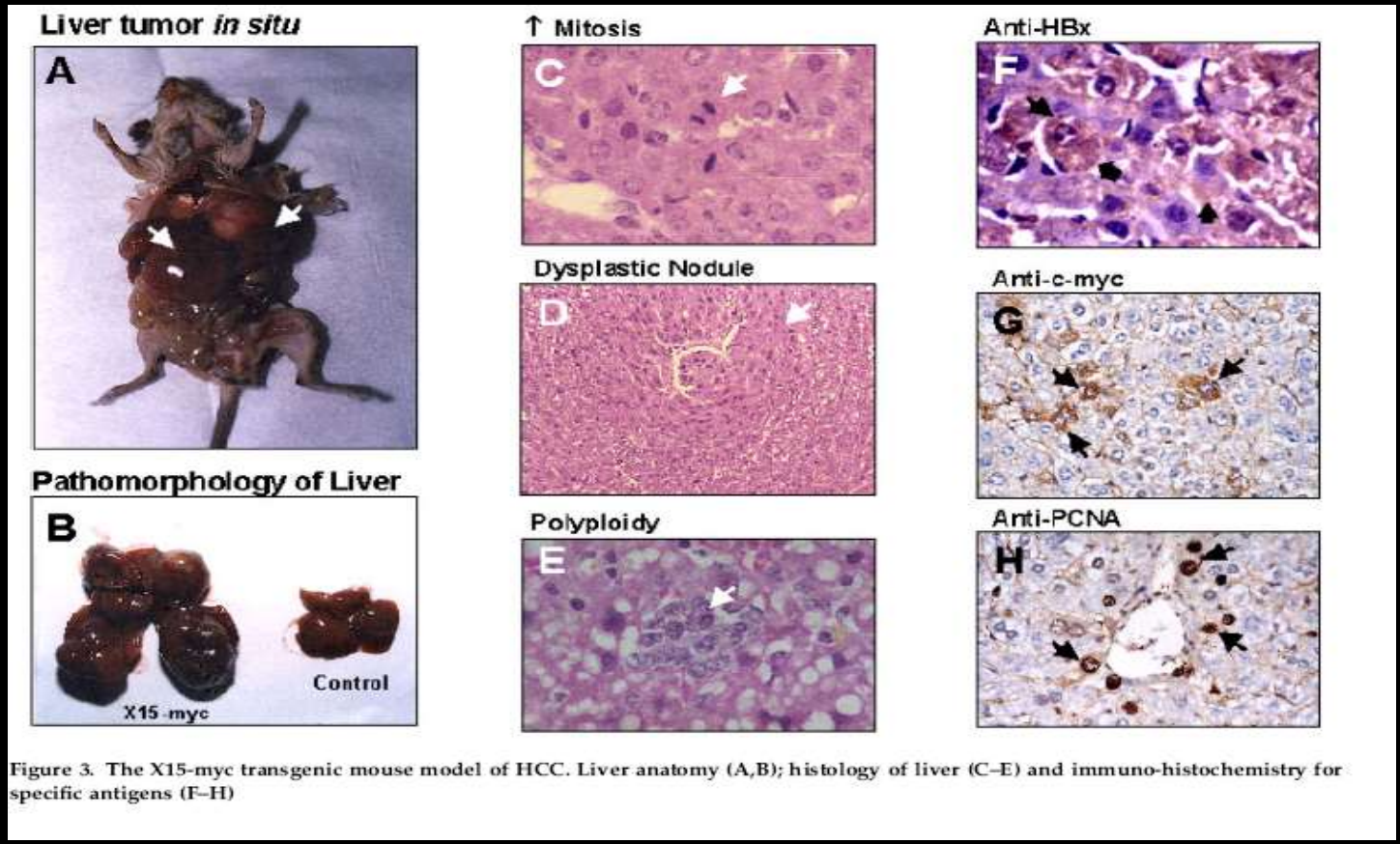
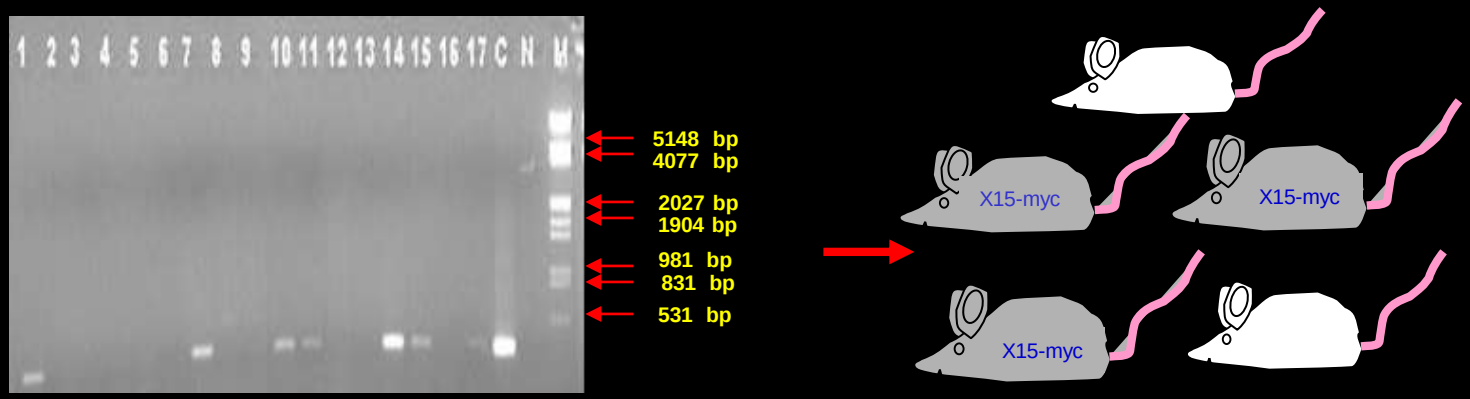


Figure 3. The X15-myc transgenic mouse model of HCC. Liver anatomy (A,B); histology of liver (C–E) and immuno-histochemistry for specific antigens (F–H)

- Most of the pathomorphological and microscopic changes were similar to those observed with the HCC patients (Lakhtakia *et al*, 2003)

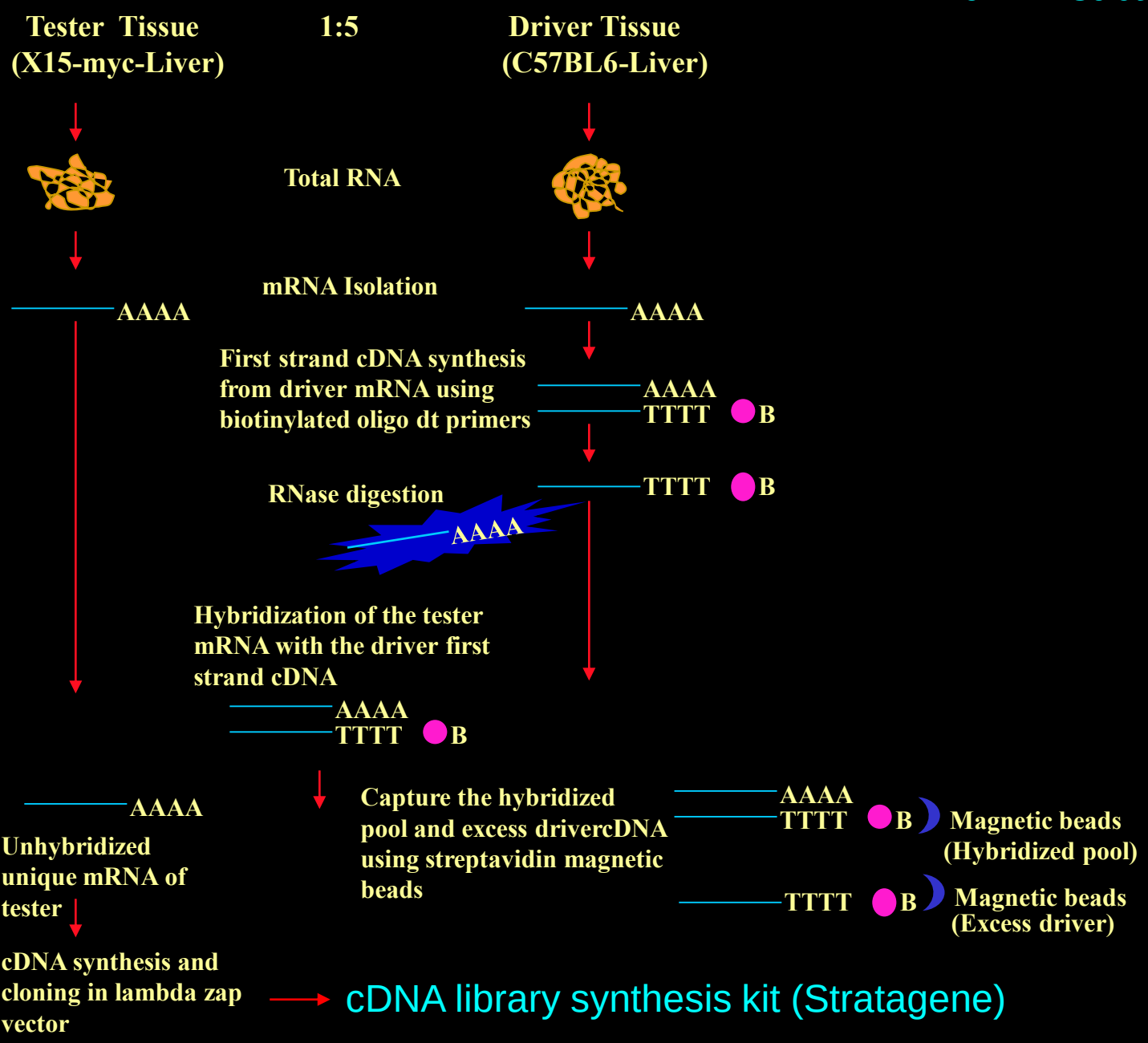
Analysis of differential gene expression in the tumors of X15-myc oncomouse.



PCR analysis for X15-myc Transgenic positive animals

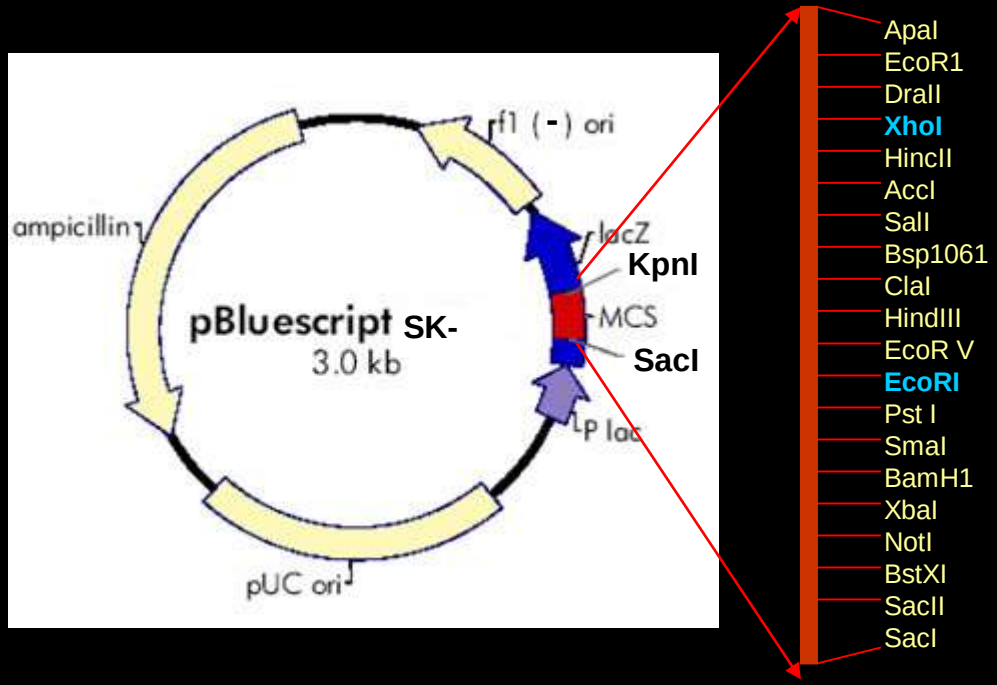
" C "- Positive control; " N" – Negative control; " M "- λ DNA Marker

cDNA subtraction

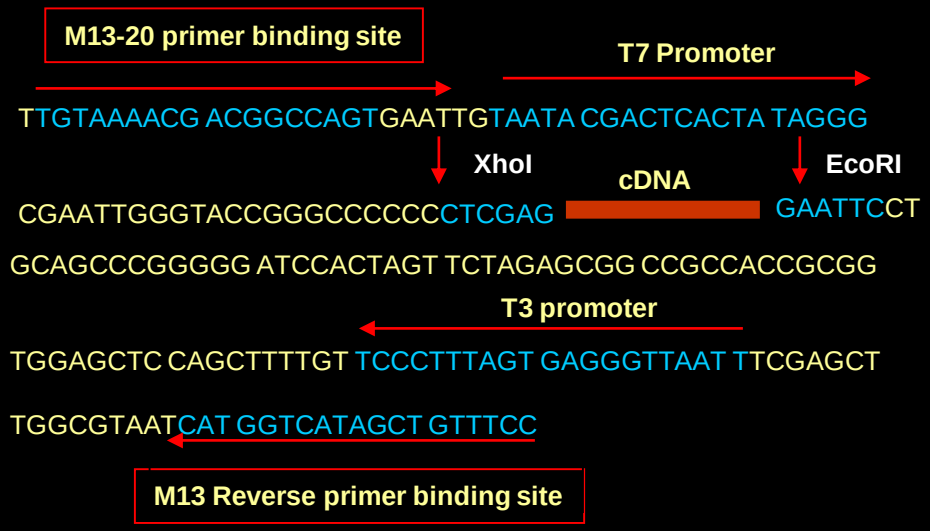


cDNA library synthesis kit (Stratagene)

A



B

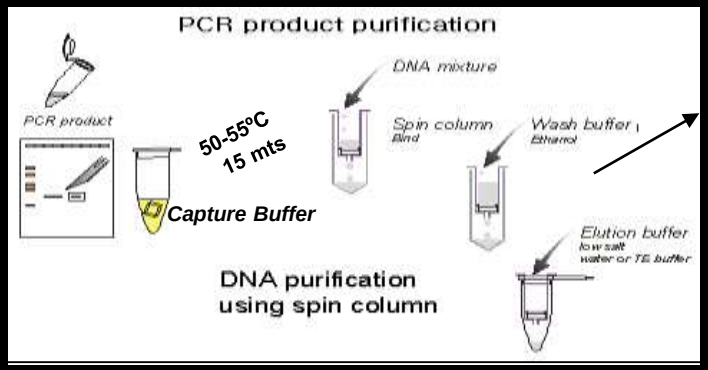


pBluescript SK(-) phagemid: A, Circular map; B, Polylinker region

Primers M13 F: 5'-CGTTGTAAAACGACGGCCAGTG -3'
M13 R: 5'-CACAGGAAACAGCTATGACCATG-3'

A.

B.



C.

A. Agarose gel analysis of PCR amplified cDNA inserts from phagemids. M1, DNA Marker, B. DNA band purification steps by GFX PCR, C. Chromotogram of MegaBACE sequencing score card.

Vector Screened & Changed in to FASTA format sequences

↓

BLAST Analysis

http://www.ncbi.nlm.nih.gov/blast/blast.cgi

List of differentially expressed genes.

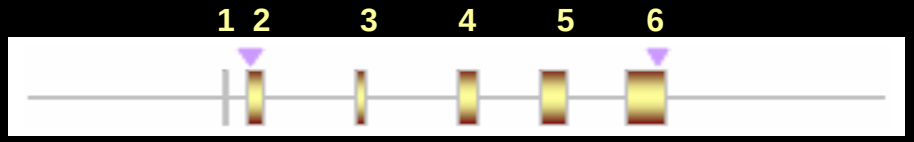
Sequence analysis of transcripts revealed 19 discreet categories

Protein biosynthesis genes	100
Electron transport genes	81
Metabolism related genes	75
Transport molecule genes	36
Ubiquitin proteasome pathway genes	25
Protein metabolism genes	23
Signal transduction genes	22
RNA processing genes	18
Calcium/sugar/carbohydrate/copper binding genes	14
Cytoskeletal prganisation/ cell adhesion genes	17
Endocytosis /protein transport genes	13
Oxidative stress genes	13
Transcription genes	13
Cell cycle and growth differentiation genes	12
Immune response genes	11
Apoptosis and anti apoptosis genes	9
Blood cogulation genes	9
Molecular chaperone genes	8
Replication gene	1

List of top five differentially expressed genes obtained from X15-myc Transgenic mouse liver cDNA subtraction library.

SYMBOL	GENE NAME	FREQUENCY
Rps27a	Ribosomal Protein s27a	20
COX3	Cytochrome C Oxidase, Subunit III	16
ATP6	ATP Synthase F0 Subunit 6	14
COX2	Cytochrome C Oxidase, Subunit II	11
ND1	NADH dehydragenase, Subunit, type 1	11

A



Exons

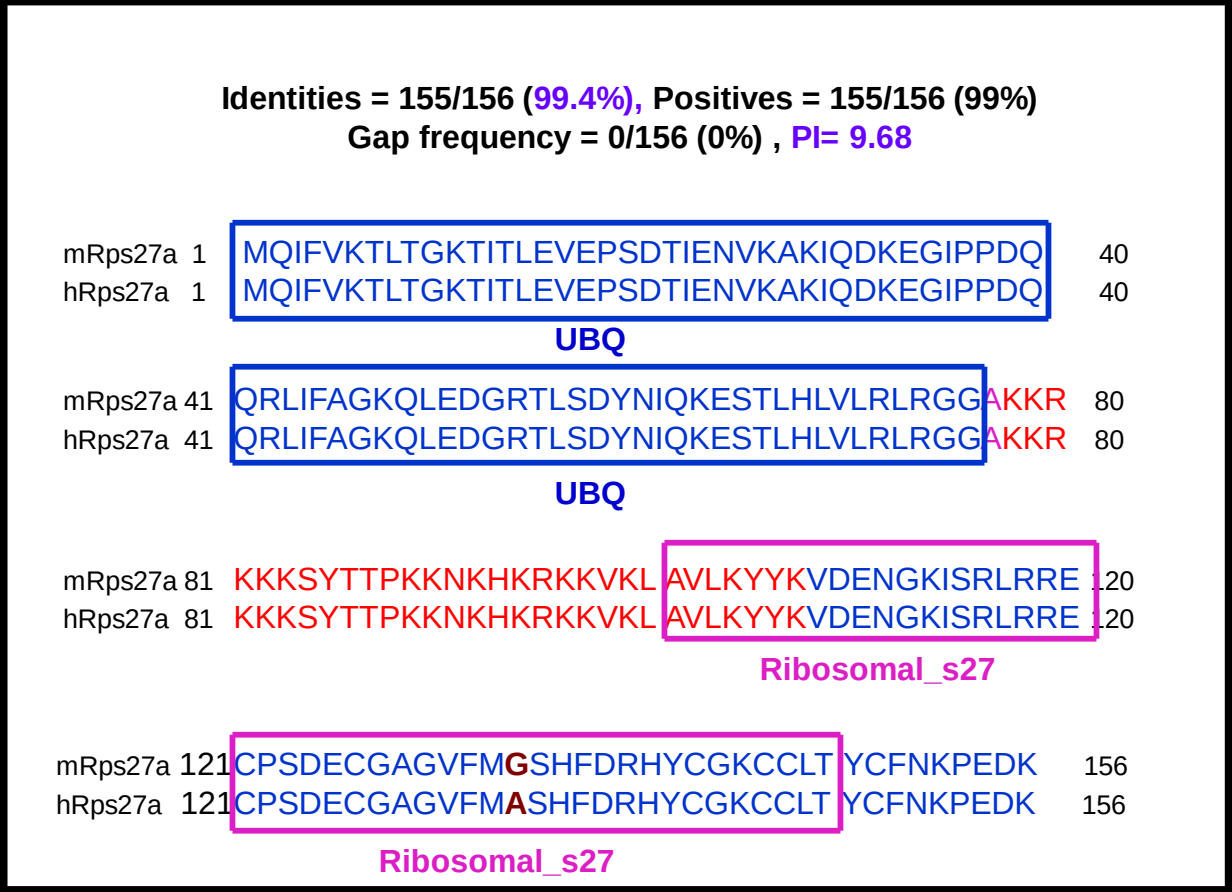
B

Chromosome: 11, Location 11 A3.3

Filename : ***Mus musculus*** **Rps27a-CDS**
Sequence Size : **471**
Translation Position : **1 - 471; Genetic Code : Universal**

```
         10        20        30        40        50        60
ATGCAGATCTTTGTGAAGACCCTTACGGGGAAAACCATCACGCTCGAGGTTGAACCCTCG
M  Q  I  F  V  K  T  L  T  G  K  T  I  T  L  E  V  E  P  S  20
         70        80        90       100       110       120
GACACTATAGAAAATGTAAAGGCCAAGATCCAGGATAAGGAAGGAATTCCTCCTGATCAG
D  T  I  E  N  V  K  A  K  I  Q  D  K  E  G  I  P  P  D  Q  40
        130       140       150       160       170       180
CAGAGGCTGATCTTTGCTGGTAAGCAGCTGGAAGATGGCCGGACTTTGTCTGACTACAAC
Q  R  L  I  F  A  G  K  Q  L  E  D  G  R  T  L  S  D  Y  N  60
        190       200       210       220       230       240
ATTCAAAAGGAGTCCACCCTTCATCTGGTGTTGAGACTTCGGGGTGGTGCTAAGAAAAGG
I  Q  K  E  S  T  L  H  L  V  L  R  L  R  G  G  A  K  K  R  80
        250       260       270       280       290       300
AAGAAGAAGTCTTACACCACTCCCAAGAAGAACAAGCATAAGAGGAAGAAGGTTAAGTTG
K  K  K  S  Y  T  T  P  K  K  N  K  H  K  R  K  K  V  K  L  100
        310       320       330       340       350       360
GCTGTGCTGAAATACTATAAGGTGGATGAAAATGGCAAAATTAGCCGACTTCGTCGAGAG
A  V  L  K  Y  Y  K  V  D  E  N  G  K  I  S  R  L  R  R  E  120
        370       380       390       400       410       420
TGTCCTTCTGATGAATGTGGTGCTGGAGTTTTCATGGGAAGCCACTTTGACAGGCATTAC
C  P  S  D  E  C  G  A  G  V  F  M  G  S  H  F  D  R  H  Y  140
        430       440       450       460       471
TGTGGCAAGTGTTGTCTGACTTACTGCTTCAACAAACCAGAAGACAAGTAG
C  G  K  C  C  L  T  Y  C  F  N  K  P  E  D  K  *        156-AA
```

Structure of the Rps27a gene of *Mus musculus* and cDNAs. **A**, Organization and chromosomal location. In total, 6 exons are shown in boxes. Closed boxes designate the coding sequences. **B**, ORF of the Rps27a gene showing 156 amino acids in single letter code (An red aerrowmark indicates the site of proteolytic cleavage that would be required to generate mature ubiquitn from the primary translation product).



Alignment of the primary sequences of human and mouse Rps27a Identical residues are shown in same color, while the non-identical regions are marked in different colors. Blue and rose boxes denote the regions of ubiquitin and Ribosomal_s27 respectively.

- The Rps27a Highly conserved very basic protein due to presence of 80 basic AA in the Carboxyl terminal (CEP) of Ubiquitin.

- UBQ-76 AA conserved eukaryotic protein- Diverse cellular functions

- UBQ-Poly ubiquitin chain (UBb, UBc) or Fused to unrelated protein (UBA)

- UBA → UBA80 (Rps27a)or UBA52 (RpL40). Conserved in man, yeast and plants

[Salvensan et al, Nucleic Acid Research (1987), Ozkaynak et al, EMBO J (1987), Lund et al, J. Bio. Chem (1985)]

- CEP analysis shows strong homology at the AA level with Zinc finger protein.

- CEP can associate with ribosome binding specifically ribosome or messenger RNA.

- Function of UBQ-CEP fusion genes has not been fully elucidated

T3- Forward Primer

5'- AATTAACCCTCACTAAAGGG -3'

A

CGGCACGAGGGCCATCGTGGGTGGAGCCGCCGCCACG**ATG**CAGATCTTTGTGAAGACCC
TTACGGGGAAAACCATCACGCTCGAGGTTGAACCCTCGGACACTATAGAAAATGTAAAGGC
CAAGATCCAGGATAAGGAAGGAATTCCTCCTGATCAGCAGAGGCTGATCTTTGCTGGTAAG
CAGCTGGAAGATGGCCGGACTTTGTCTGACTACAACATTCAAAAGGAGTCCACCCTTCATC
TGGTGTTGAGACTTCGGGGTGGTGCTAAGAAAAGGAAGAAGAAGTCTTACACCACTCCCA
AGAAGAACAAGCATAAGAGGAAGAAGGTTAAGTTGGCTGTGCTGAAATACTATAAGGTGGA
TGAAAATGGCAAAATTAGCCGACTTCGTCGAGAGTGTCCTTCTGATGAATGTGGTGCTGGA
GTTTTCATGGGAAGCCACTTTGACAGGCATTACTGTGGCAAGTGTTGTCTGACTTACT**GCT**
TCAACAAACCAGAAGGAACAAAGGTTAGGTTTAGGTGTATGCCCGTTTAAATTAAACAGGA
ACGGAAACTTCGTTCAANCAAACGACAGAACNGACGACAAAAACAACACTCCGGGGGGGG
GGGGGGGCCGGGACACCAATTCCACCTAATGNGATCTGTAATCAAGNATCGCGGGGCCGA
GTCTAACAAGGGGATGCCACCGAGCAGGCAGAAGAGGGGGAGNAGACTAACNACACAAC
ATGCATGAGGAGGCAGATGAGGGGANACCGGATATCTGTGNCGTNNCCACCGCTTCCAGC
ACACACGTTCGTGNACTCAGGNTNCNCNCNTTTGTCNNT

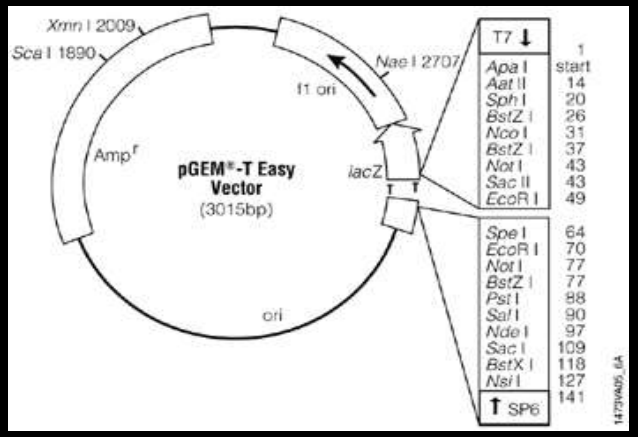
Reverse Primer **Bam H1**

3'

CGAAGTTGTTTGGTCTTCTGTTCATCCCTAGGGC

5'

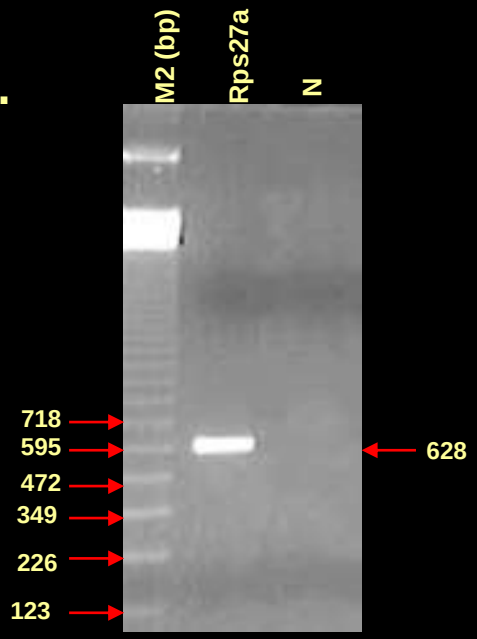
B



Cloning of the Rps27a ORF. **A**, Phage template 1173 showing the location of primers for PCR amplification (Reverse primer binding regions are underlined).

B. pGEMT easy vector: Circular map

A.



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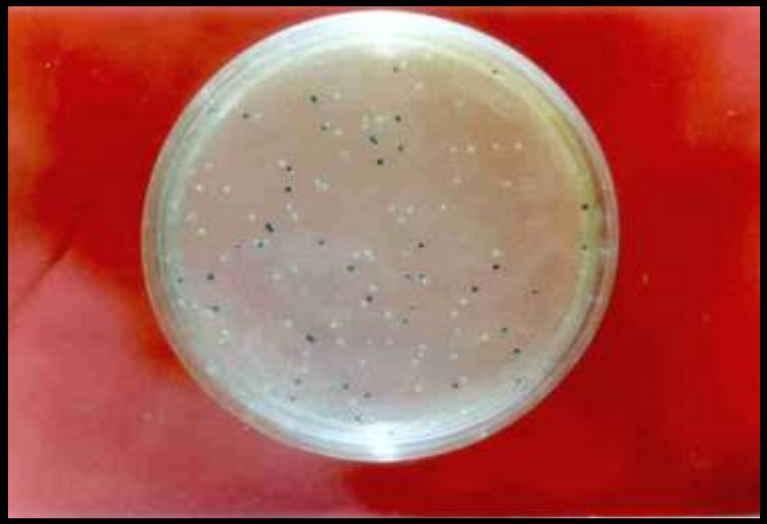
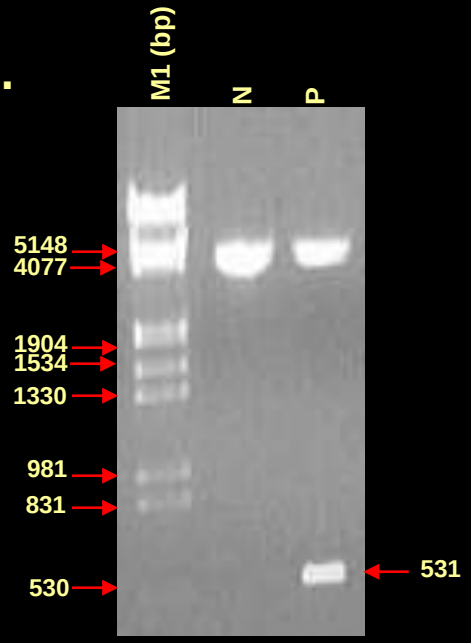


C.



Cloning of the Rps27a ORF. **A,** PCR products shown in agarose gel
B, Blue white colony selection
C, Positive clones in pGEMT easy vector digested with BamHI.
M1, DNA marker; **M2**, 123 bp marker; **N**, Negative Control; **P**, Positive clone.

A.

CEP 1 (245 bp) - 78 156AA

CEP 2 (191 bp) - 101 156AA

Rps27a (471-nt)-Template

ATGCAGATCTTTGTGAAGACCCTTACGGGGAAAACCATCACGCTCGAGGTTGAACCCTCGG
ACACTATAGAAAATGTAAAGGCCAAGATCCAGGATAAGGAAGGAATTCCTCCTGATCAGCAG
AGGCTGATCTTTGCTGGTAAGCAGCTGGAAGATGGCCGGACTTTGTCTGACTACAACATTCA
AAAGGAGTCCACCCTTCATCTGGTGTTGAGACTTCGGGGTGGTGCTAAGAAAAGGAAGAAG
AAGTCTTACACCACTCCCAAGAAGAACAAGCATAAGAGGAAGAAGGTTAAGTTGGCTGTGCT
GAAATACTATAAGGTGGATGAAAATGGCAAAATTAGCCGACTTCGTCGAGAGTGTCCTTCTG
ATGAATGTGGTGCTGGAGTTTTCATGGGAAGCCACTTTGACAGGCATTACTGTGGCAAGTGT
TGTCTGACTTACTGCTTCAACAAACCAGAAGACAAGTAG

PCR-PRIMERS

EcoRI

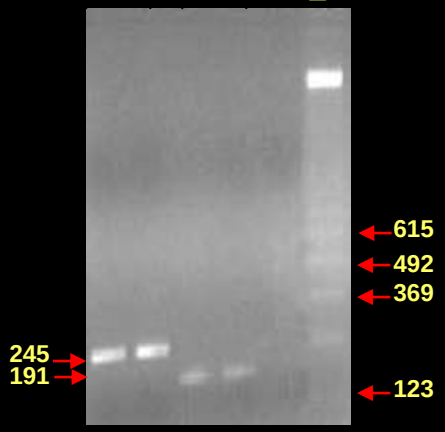
CEP1- F 5' CGGAATTCATGGCTAAGAAAAGG 3'

CEP2- F 5' CGGAATTCATGGCTGTGCTGAAATAC

XhoI

CEP1 & 2 R 5' CCCTCGAGCTTGTCTTCTGG 3'

B.



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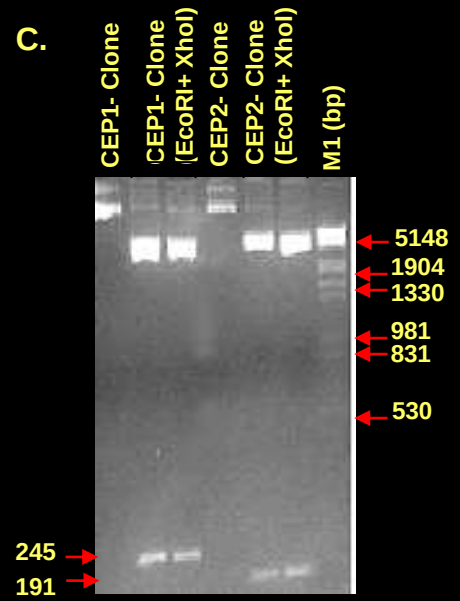
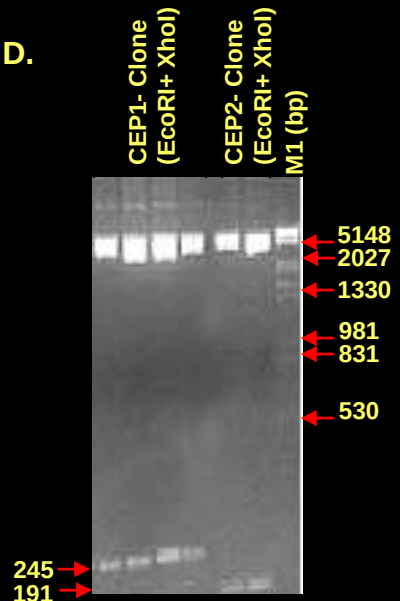


D.



Construction of prokaryotic and eukaryotic expression vector for carboxyl extension protein (CEP)

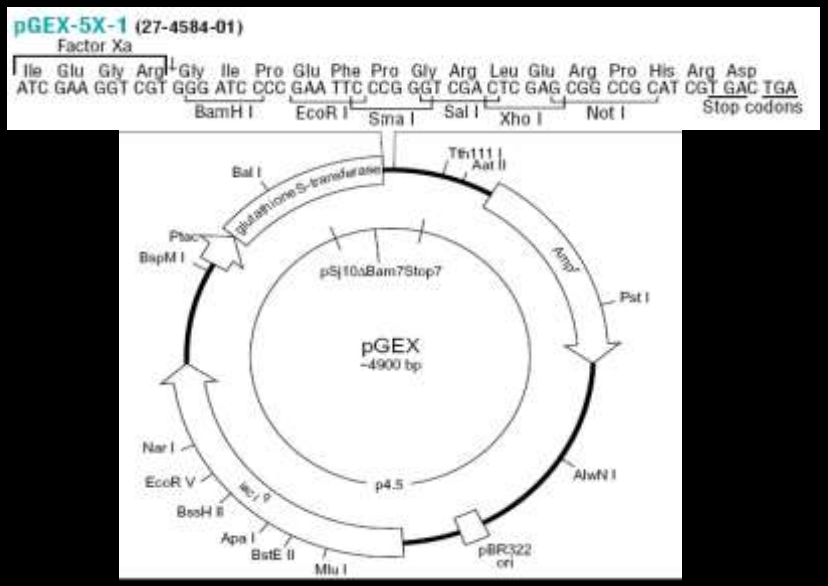
A, The gene coding for 78-156 AA (CEP1) and 101-156 AA coding for CEP2 are shown

B, PCR amplified fragments of CEP1 and CEP2

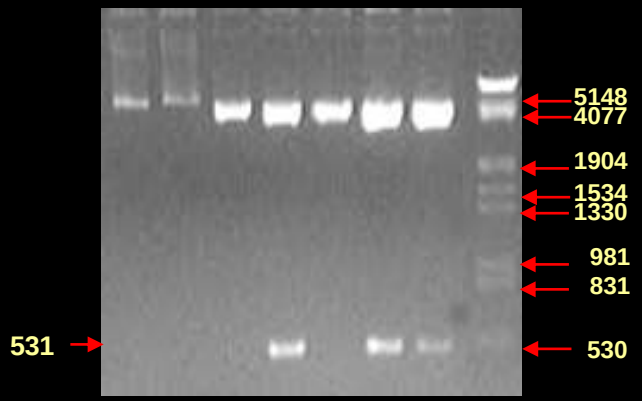
C, Positive clone of CEP1 and CEP2 in pGEX5X1 vector digested with EcoRI and XhoI

D, Positive clone in pSG5 vector digested with EcoRI and XhoI. **M1**, DNA marker; **M2**, 123 bp marker.

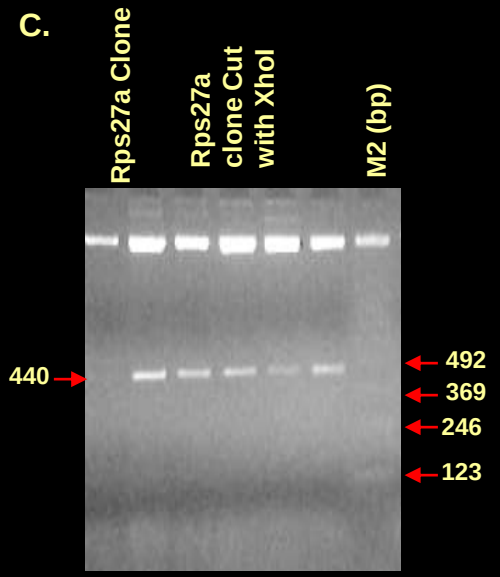
A.



B.



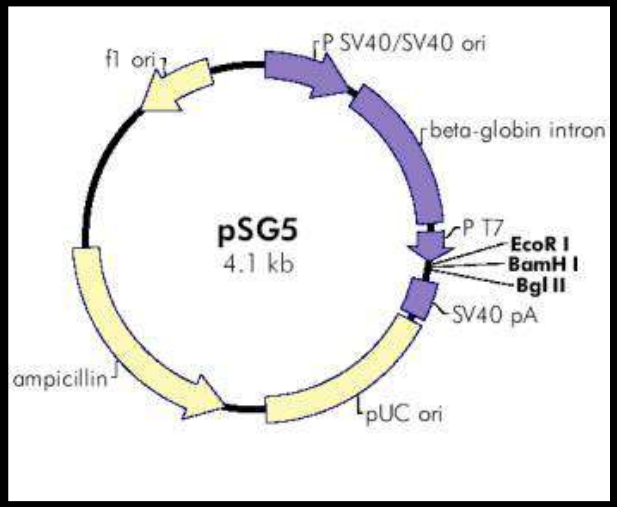
C.



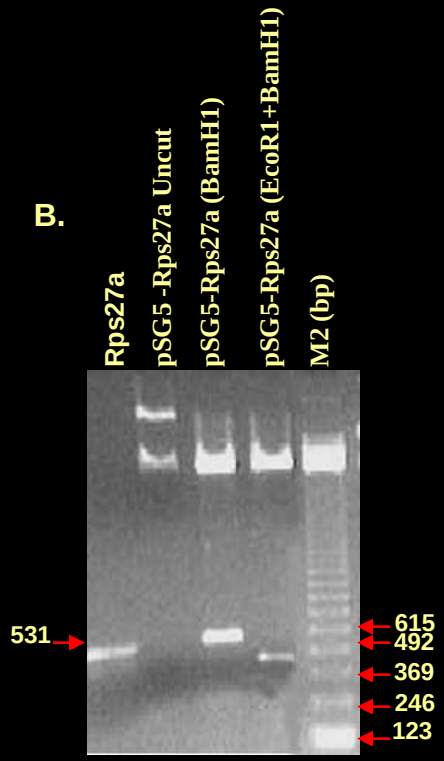
Construction of prokaryotic expression vector for Rps27a.

A, pGEX5X1 plasmid vector map
B, Positive clone in pGEX5X-1 vector digested with BamHI
C, Screening the orientation (Right) pattern.
M1, DNA marker; **M2**, 123 bp marker.

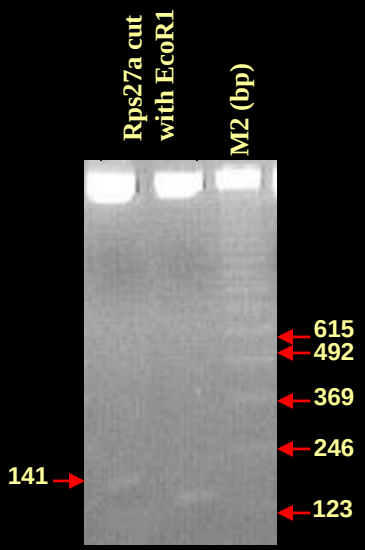
A.



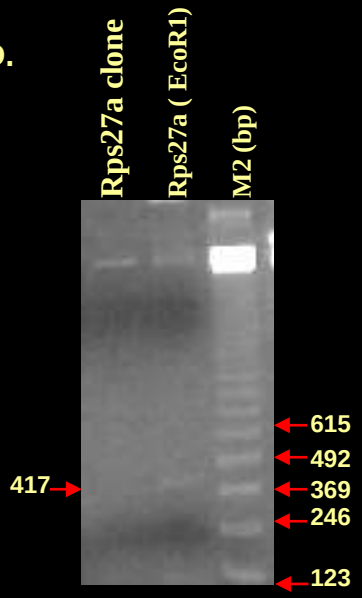
B.



C.



D.



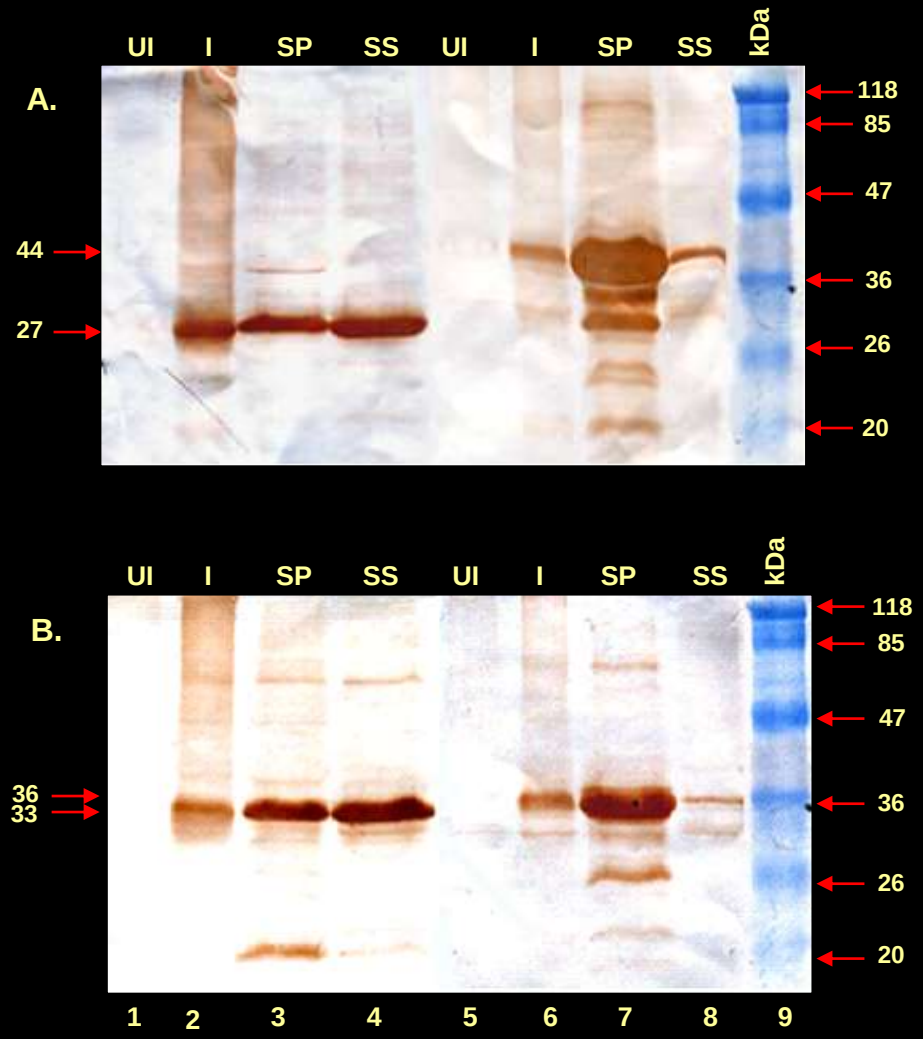
Construction of eukaryotic expression vector for Rps27a.

A, pSG5 plasmid vector map

B, Positive clone in pSG5 vector digested with BamHI

C, Right orientation of Rps27a Clone cut with EcoRI

D, Wrong orientation of Rps27a clone cut with Rps27a. **M2**, 123 bp marker.

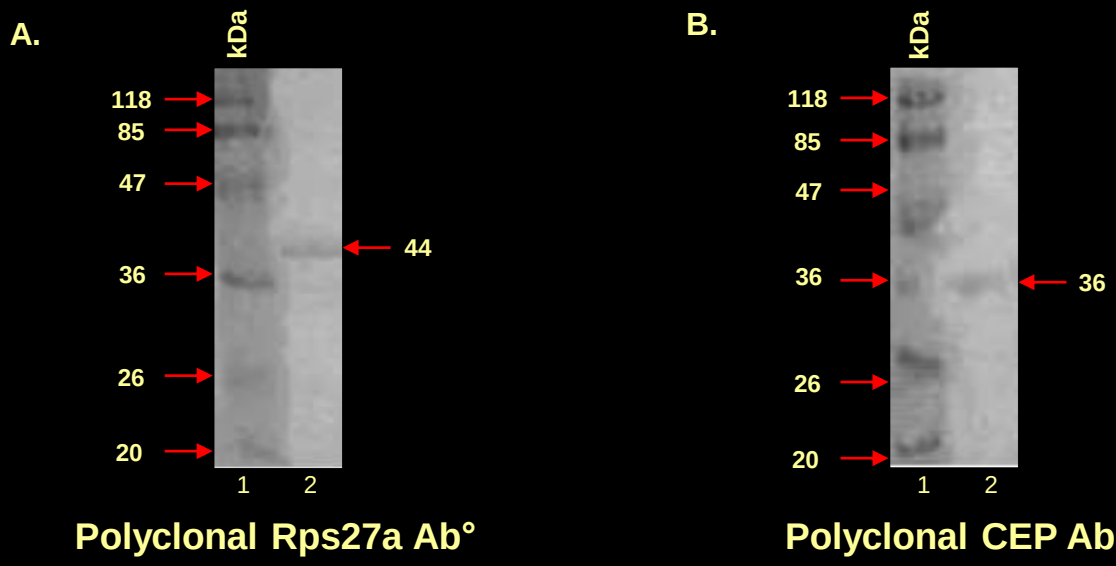


Western blot analysis of GST-Rps27a and GST-CEP recombinant protein expression in *E.coli.*

(A) IPTG (1mM) induced both crude and sonicated GST (27 kDa) and recombinant GST-Rps27a (44 kDa) protein

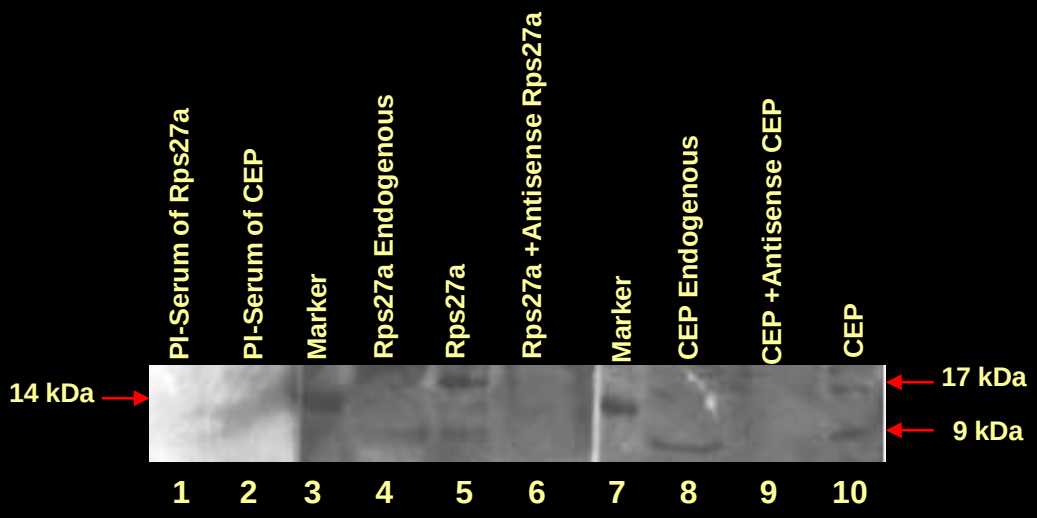
(B) GST-CEP1 (36 kDa) protein and GST-CEP2 (33 kDa) protein extracts were probed with GST antibody. UI, Un-induced; I, Induced; SP, Sonicated Pellet; SS, Sonicated Supernatnt.

Authentication of Recombinant Rps27a and CEP proteins

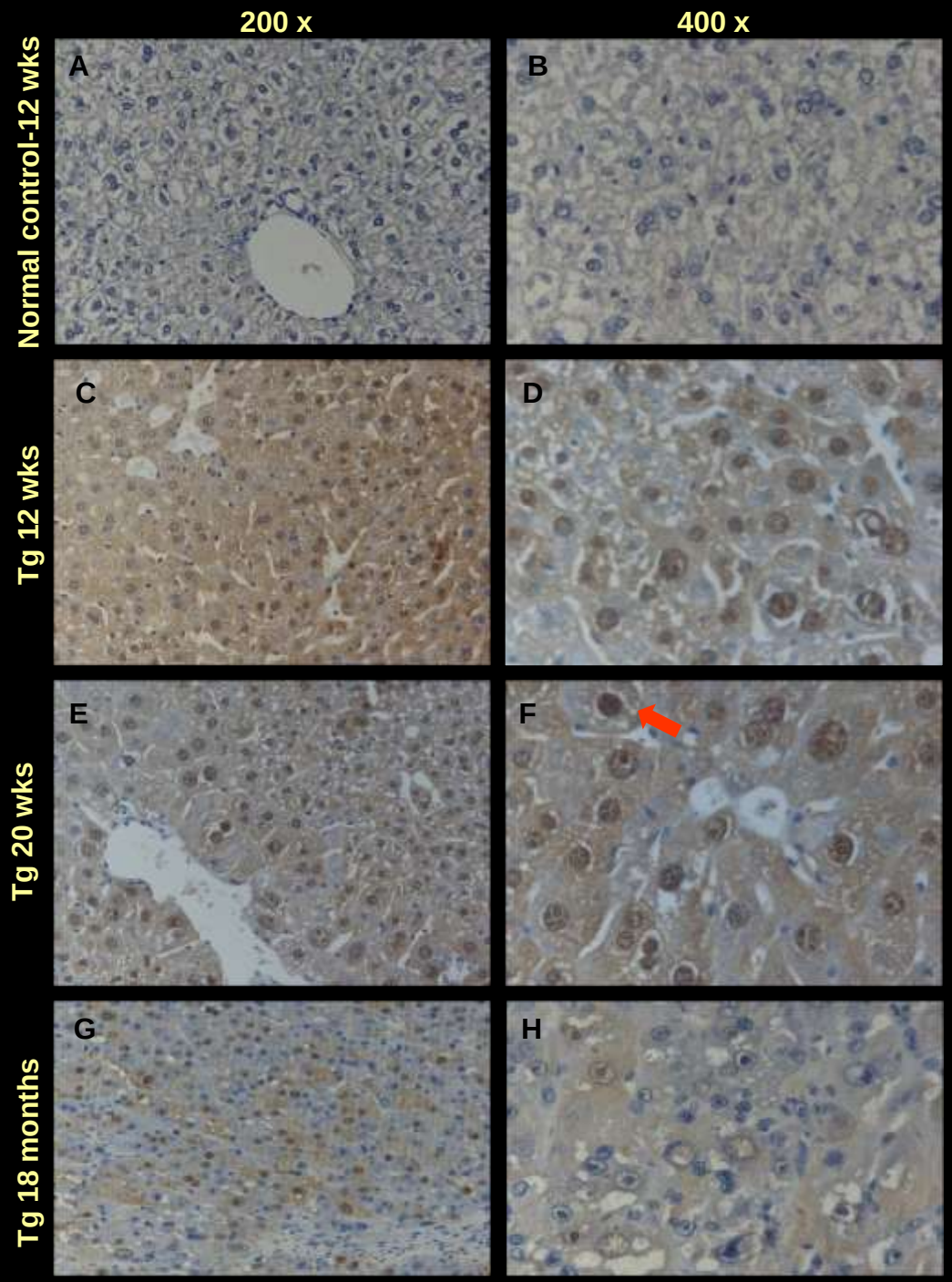


Western blot analysis GST-Rps27a and GST-CEP

E.coli **expression of recombinant GST-Rps27a (A) and GST-CEP (B) proteins were transferred on to nitrocellulose strips and probed with anti-Rps27a (1:200) (A. Lane 2) and anti-CEP polyclonal sera (B. Lane 2). Antigen- antibody reaction was shown on RH side arrow mark. kDa, Prestain marker.**



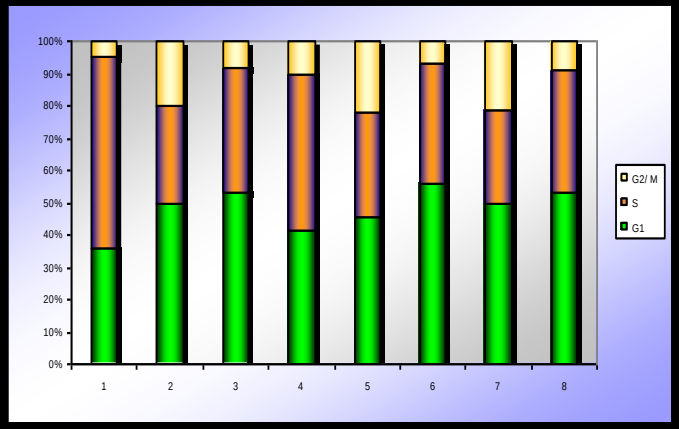
Detection of Rps27a and CEP expression in Huh7 cell lines.
Total cells were lysed after 48hr posttransfection with Rps27a (lanes 5), anti-Rps27a (lanes 6), CEP (lanes10) and anti-CEP (lanes 9).



Rps27a expression in X15-myc Tg mice liver tissues. 12 wks **(C&D),** 20 wks **(E&F),** 18 months old **(G&H)** Tg mice and 12 wks old **(A&B)** normal mice (control) liver sections were stained with anti- Rps27a monoclonal antibody, the 80 amino acid extension of CEP80. Positive staining is noted more in nuclear **(red arrow**) and cytoplasmic regions **(C)** of Tg mouse liver sections **(C,D,E,F,G &H). (A,C, E& G = x200 and B,D,F& H = x400)**

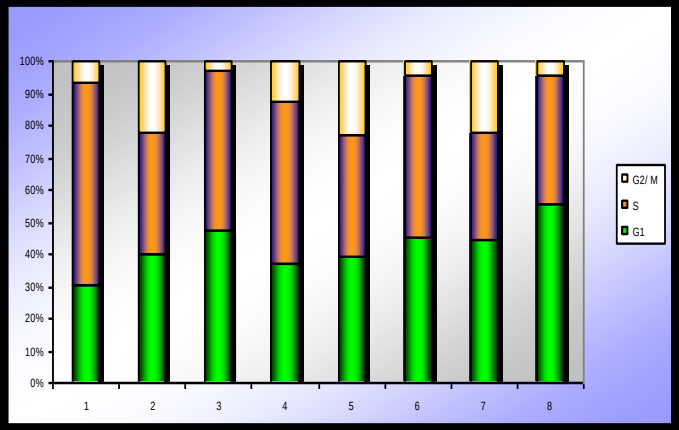
A

12hrs



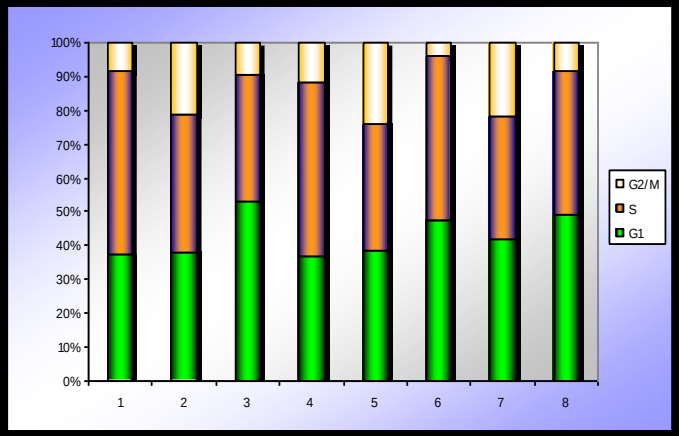
B

24hrs



C

42 hrs



Constructs

1. Rps27a
2. Antisense Rps27a
3. Rps27a + Antisense Rps27a
4. CEP
5. Antisense CEP
6. CEP + Antisense CEP
7. Rps27a + Antisense Rps27a +Antisense CEP
8. pSG5

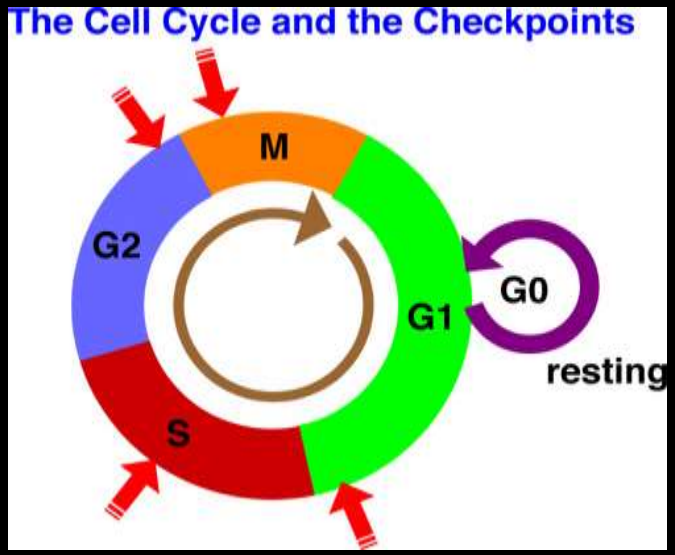
Effect of Rps27a and CEP on cell cycle. Huh7 cells were transfected with Rps27a, CEP, anti-Rps27a and anti-CEP constructs at 60-70 % confluency and cells were serum syncronized and collected at various time points of 12hrs (A) , 24hrs (B) and 42 hrs (C). The percentages of cells at different phases of the cell cycle were analysed by FACS and values are shown on the left (Y-axis) bar diagram.

Report-Deletion Rps6 (40s RS) ⊣ Cell proliferation

(Rps27a & CEP) ↓

G2/M phase arrest

Cyclin B1
(Yu et.al., 2002)



Cyclin E (sherr et.al.,1999)

Rps27a- early growth response gene G1 phase cell division

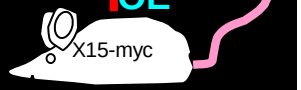
Induction of
Cyclin D-CDK4
Cyclin D-CDK2

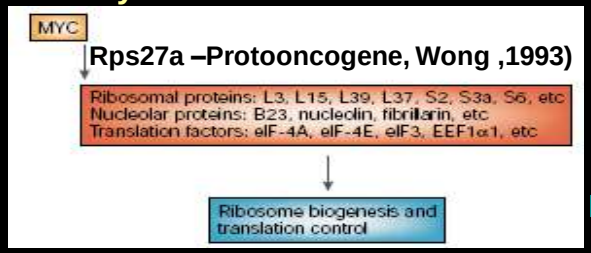
"S"PHASE ↑
DNA replication

(Rps27a & CEP) ↑

OE

OE





DeBenedetti *et al.*, 1990

In yeast, UB13 deletion-Slow growth of phenotype (1.6 hr-6.8hr) (Finley et.al., 1989)
Many small & Large Rib.Sub unit Protein ↑ variety of p.Tumor- Ex RPL36A. OE-Over Expression

Summary

- Rps27a, a ubiquitin precursor protein fused to the 80 amino acid carboxyl extension protein (CEP)

- The flow cytometry analysis reveled that Rps27a overexpressing cells accelerated cells to enter G1 phase to S phase with enhanced cell proliferation

- Immunohistochemical staining of Rps27a expression was moderately reduced from 3 months to 18 months old Transgenic liver tissues.

Overall, The sensitivity of over expressed Rps27a in HCC might be a general biomarker for tumor proliferation.

Evaluation of the antitumorogenic/anti-angiogenic activities of the floral isolates of *B. monosperma* in the transgenic mouse model.

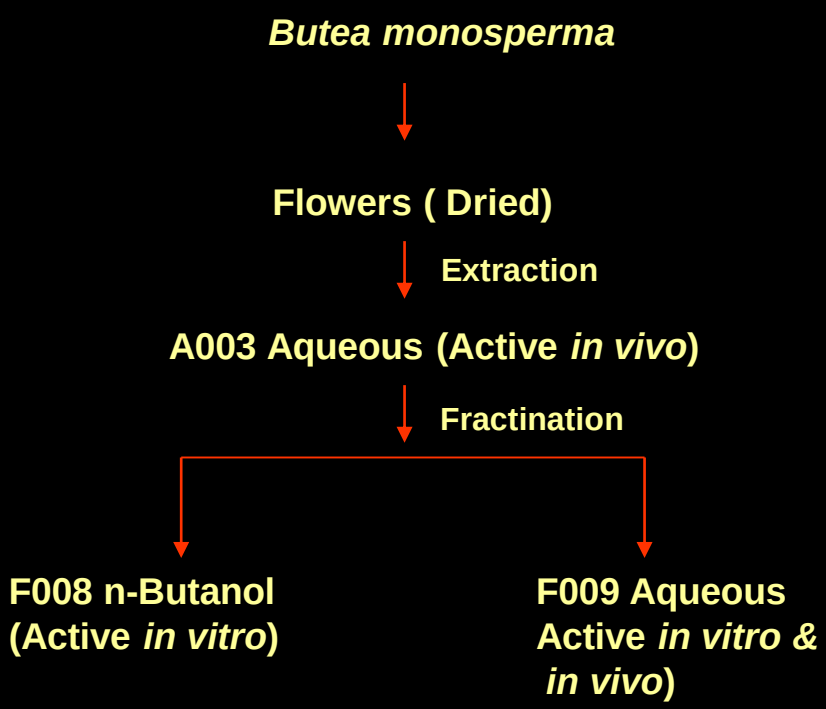
Hepatoprotective Herbal Extracts

- **Tredational Herbal Medicine & CAM becoming popular among cancer patients (Molassiotis *et al*., 2005; Yates *et al*, 2005)**

- **RRL (Regional Research Laboratory, CSIR), Jammu (Collection of medicinal herbs from all over India)**

Test Material	A003	F008	F009	Adriamycin	Mitomycin	Tamoxifen	5-Flurouracil
Concentration used:	100 mg/L	100 mg/L	100 mg/L	$1x10^{-5}$ M	$1x10^{-5}$ M	$1x10^{-5}$ M	$2x10^{-5}$ M
Cell lines							
Breast – MCF-7	6	-	-	72	-	-	-
Breast – T47D	4	22	0	34	-	-	-
Breast – ZR75-1	0	-	-	46	-	-	-
Cervix – SiHa	0	9	0	-	-	-	19
CNS - SK N MC	23	57	2	-	-	27	-
CNS - SK N SH	43	-	-	82	-	-	-
CNS - SNB78	2	-	-	20	-	-	-
Colon - Colo205	87	19	0	-	-	-	40
Liver - Hep2	51	58	35	-	88	74	-
Lung - A549	19	27	11	58	-	17	-
Lung - NCI H23	0	-	-	-	59	-	-
Oral - KB	16	-	-	-	-	-	9
Ovary – NIH OVCAR3	0	-	-	-	31	-	-
Ovary – OVCAR5	5	37	6	-	-	-	-
Prostate – DU145	0	-	-	-	69	-	-

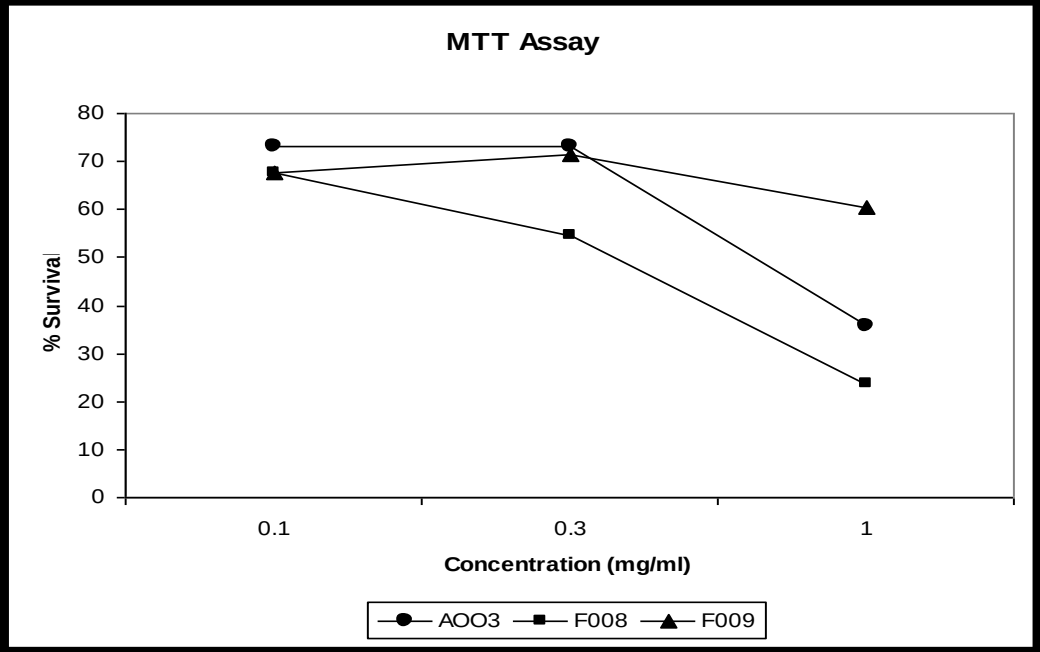
Percent growth inhibition of human cancer cell lines with the aqueous extract (A003) of flowers of *Butea monosperma* and its fractions (F008 and F009) [Mathan *et.al.*,] [Communicated]



Flowchart of *Butea monosperma* floweral extracts and its fractions.

Butea monosperma (Flame of the Forest)

Family	:	Fabaceae
Orgin	:	India
Type/Uses	:	flowering tree
Size	:	50feet
Growth Rate	:	Slow growing at first
Lighter Requirments	:	full sun
Water Requirments	:	average, drier in the winter
Min.Temp.	:	mid 30° s
Flower	:	late winter, spring



Cytotoxic effect of *Butea monosperma* extract on cancer cell lines. **Huh-7 cells were incubated with different concentrations of *Butea monosperma* and analyzed for cell viability at 24h or 48h (*n*=6), mean±SE. a.*P*<0.001; b. *P*<0.01**

- **IC_{50} in Huh7 cells – F009- less toxic (65% cell survival at 1g/L)**
  - **A003 & F008 (0.5 – 1g/L) & Doxorubicin (0.1g/L)**

X 15-myc Transgenic mouse model

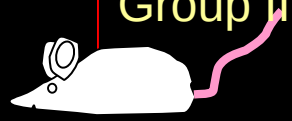
(4 weeks old matched age group, n=6)

Group I

12 weeks

Receives 9 ip doses of A003, F008 & F009 in saline on biweekly (100 mg/kg)

Group II



20 weeks

Serum VEGF
Tumor weight
Histopathology
Immunohistochemistry for Rps27a
Morphometry

Anti- tumorigenic effect of *B. monosperma* in transgenic mice

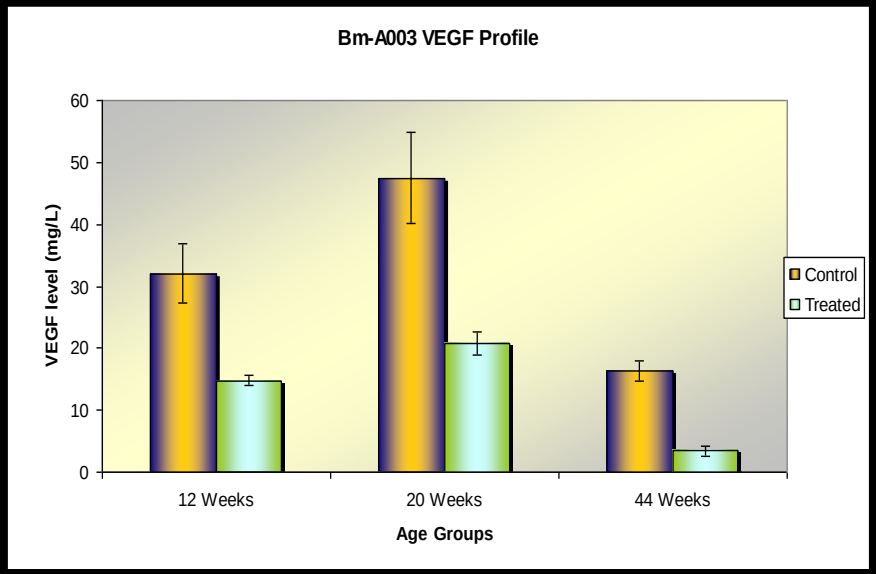
A.

	Period (wks)	Group: Treated(n=6)	Group: Control (n=6)
A003	12	14.8±0.8[b]	32.1±4.8
	20	20.8±1.9[a]	47.5±7.3
	44	3.45±0.8[a]	16.3±1.6
F009	12	13.5±0.6[NS]	14.4±0.5
	20	16.2±0.6	17.1±0.8[NS]
Non transgenic control		7.00 ± 0.55	

Values are shown as mean± SE. [a] p<0.001; [b] p<0.01; [NS] p = 0.15

Tg-2 to 4 fold↑

B.



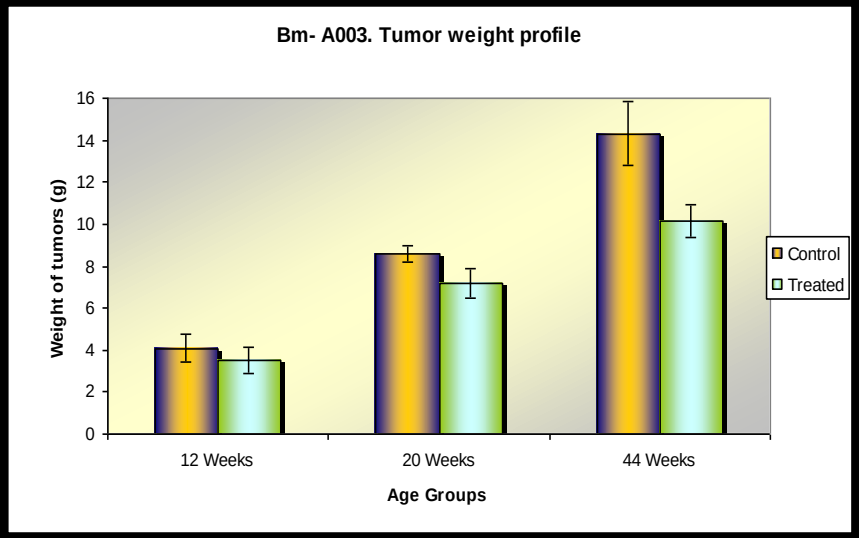
A. Serum VEGF levels (mg/L) in the X-15 *myc* mice after treatment with the aqueous extract (A003) or aqueous fraction (F009) of *Butea monosperma (BM).* B. BM-A003 treated, 20 and 44 wks old, Transgenic animals are showing the significant reduction (p<0.001) in their VEGF level.

A.

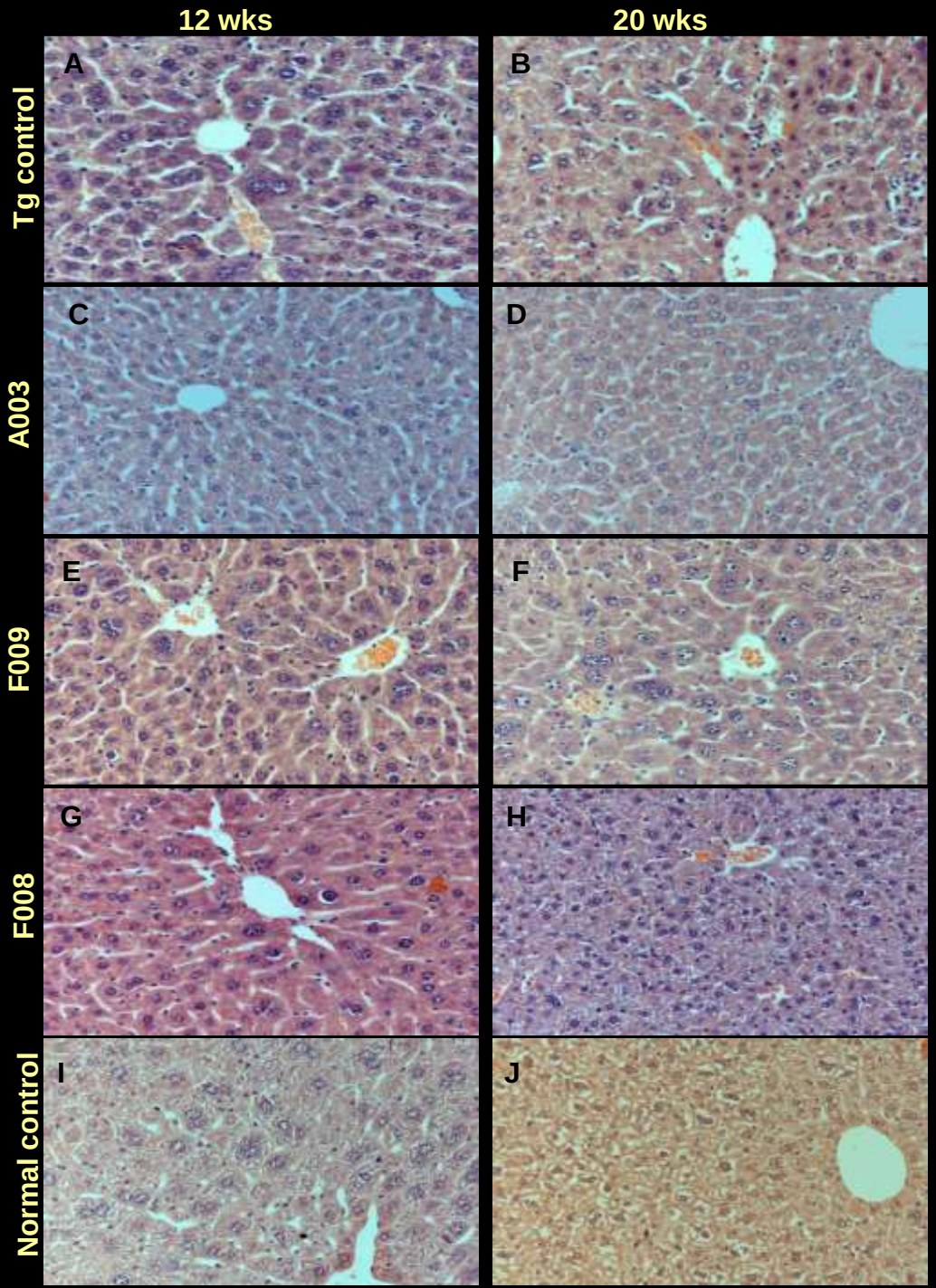
	Period (wks)	Group: Treated(n=6)	Group: Control (n=6)
A003	12	3.50±0.63	4.08±0.66
	20	7.17±0.72[b]	8.57±0.39
	44	10.15±0.81[a]	14.32±1.53

Values are shown as mean± SE. [a] p<0.001; [b] p<0.01

B.

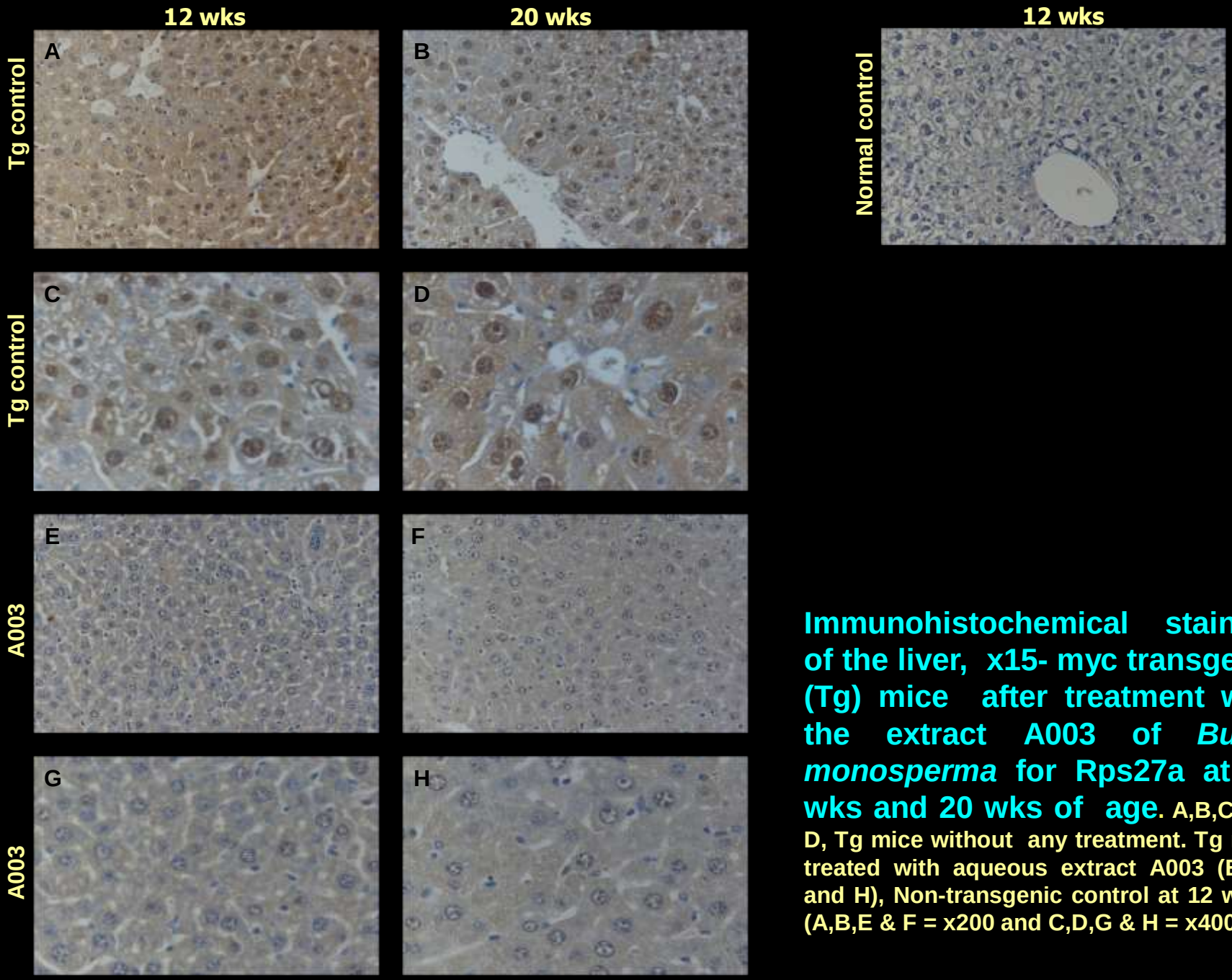


A. Liver tumor weight (g) of X-15 *myc* mice after treatment with the aqueous extract (A003) of *Butea monosperma (BM).* B. BM-A003 treated, 44 wks old, Transgenic animals are showing the significant reduction (p<0.001) in their tumor weight.



Histological analysis of the liver of X15-*myc* transgenic (Tg) mice at 12 wks and 20 wks of age after treatment with the extract and fractions of *Butea monosperma*.

(A&B), Tg mice without any treatment. Tg mice treated with aqueous extract A003 **(C&D),** aqueous fraction F009 **(E&F)** and butanolic fraction F008 **(G&H); (I&J),** Non-transgenic control at 12 wks and 20 wks. **(All x200)**



Immunohistochemical staining of the liver, x15- myc transgenic (Tg) mice after treatment with the extract A003 of *Butea monosperma* for Rps27a at 12 wks and 20 wks of age. A,B,C and D, Tg mice without any treatment. Tg mice treated with aqueous extract A003 (E,F,G and H), Non-transgenic control at 12 wk (I) (A,B,E & F = x200 and C,D,G & H = x400)

12- wks

Parameters	Treatment Groups				
	Normal Control	Tg Control	A003	F008	F009
Area	26.9±11.8[a]	45.54±22.0	22.7±10.6[a]	42.46±26.4[c]	42.36±23.6[c]
Diameter(mean)	5.53±1.20[a]	7.26±1.81	5.00±1.30[a]	6.84±2.10[a]	6.90±1.93[a]
Perimeter	18.05±3.72[a]	24.18±6.89	16.40±4.10[a]	22.26±6.75[a]	22.50±6.50[a]
Size (length)	6.20±1.45[a]	8.60±2.45	5.60±1.40[a]	7.60±2.34[a]	7.65±2.20[a]
Size (width)	5.24±1.00[a]	6.50±1.64	4.80±1.25[a]	6.53±1.98	6.50±1.85
Feret (mean)	5.80±1.18[a]	7.62±2.00	5.22±1.30[a]	7.09±2.13[a]	7.14±2.00[a]
Density(mean)	124.7±10.8[a]	119.43±11	132.22±9.8[a]	108.7±12.3[a]	126.43±15[a]
Density (std.dev)	13.83±2.63[a]	16.03±4.43	17.00±4.20	18.40±6.62[a]	20.45±7.16[a]
Heterogeneity	0.064±0.05[a]	0.109±0.1	0.149±0.10[a]	0.154±0.12[a]	0.209±0.14[a]

Values are shown as mean± SE (n=500).**'a' = p<0.001; 'b' = p<0.01; 'c' = p<0.05.**

Mean values and standard deviation for the parameters of area, diameter (mean), perimeter, size (length & width), feret (mean) Density (mean), density (std.dev) and heterogeneity (in millimeter) of the liver cell nuclei of 12 wks old, X15- myc transgenic mice treated with *Butea monosperma* (A003) and its fractions (F008 and FOO9) with their relevant Tg control.

20- wks

Parameters	Treatment Groups				
	Normal Control	Tg- Control	A003	F008	F009
Area	26.85±11.8[a]	47.96±24.6	23.95±15.0[a]	26.85±14.5[a]	41.64±24.6[a]
Diameter(mean)	5.526±1.20[a]	7.370±1.90	5.071±1.60[a]	5.439±1.52[a]	6.760±2.10[a]
Perimeter	18.04±3.72[a]	24.20±6.24	16.56±5.17[a]	17.84±4.94[a]	22.14±6.91[a]
Size (length)	6.194±1.45[a]	8.433±2.32	5.669±1.75[a]	6.230±1.75[a]	7.567±2.40[a]
Size (width)	5.237±1.00[a]	6.879±1.70	4.844±1.60[a]	5.088±1.46[a]	6.395±2.00[a]
Feret (mean)	5.763±1.18[a]	7.684±1.95	5.286±1.64[a]	5.691±1.56[a]	7.039±2.15[a]
Density(mean)	124.7±10.8[a]	113.52±13	122.4±10.2[a]	105.6±17.2[a]	121.7±11.1[a]
Density (std.dev)	13.83±2.63	14.25±4.35	17.86±5.03[a]	21.83±8.54[a]	16.44±4.26[a]
Heterogeneity	0.064±0.05	0.073±0.07	0.160±0.12[a]	0.198±0.16[a]	0.126±0.10[a]

Values are shown as mean± SE (n=500). **'a' = p<0.001; 'b' = p<0.01; 'c' = p<0.05.**

Mean values and standard deviation for the parameters of area, diameter (mean), perimeter, size (length & width), feret (mean) Density (mean), density (std.dev) and heterogeneity (in millimeter) of the liver cell nuclei of 20wks old, X15- myc transgenic mice treated with *Butea monosperma* (A003) and its fractions (F008 and FOO9) with their relevant Tg control.

SUMMARY

- *in vitro* cytotoxicity and *in vivo* transgenic animal studies suggested that aqueous extract (A003) and fractions (F008 and F009) of *Butea monosperma* flowers is not only hepatoprotective but also carries anti-proliferative, anti-tumorogenic and anti-angiogenic properties.

- The chemopreventive action of fractions, F008 and F009 was less prominent as compared to the aqueous extract A003 suggesting either loss or inactivation of some of the key constituents in these fractions.

- Immunohistochemical analysis of Rps27a in the transgenic animals treated with aqueous extract A003 revealed a marked reversal of pathological manifestation including no staining for Rps27a in the liver.

Overall, the aqueous extract of *Butea monosperma* flower has the potential for developing new cancer therapeutics

PATENTS

1. Saxena, A. K., Gupta, B. D., Kapahi, B. K., Shanmugavel, M., Mondhe, D. M., Qazi, G. N., **Mathan, G.,** and Kumar, V. Anticancer activity of an extract from *Butea monosperma* and the fraction thereof. Patent application #20060280817; Class: 424757000 (USPTO), 2006.

Thanks for your Attention

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