



H19 REGULATED GENES AND MiRNAs IN MENINGIOMA:

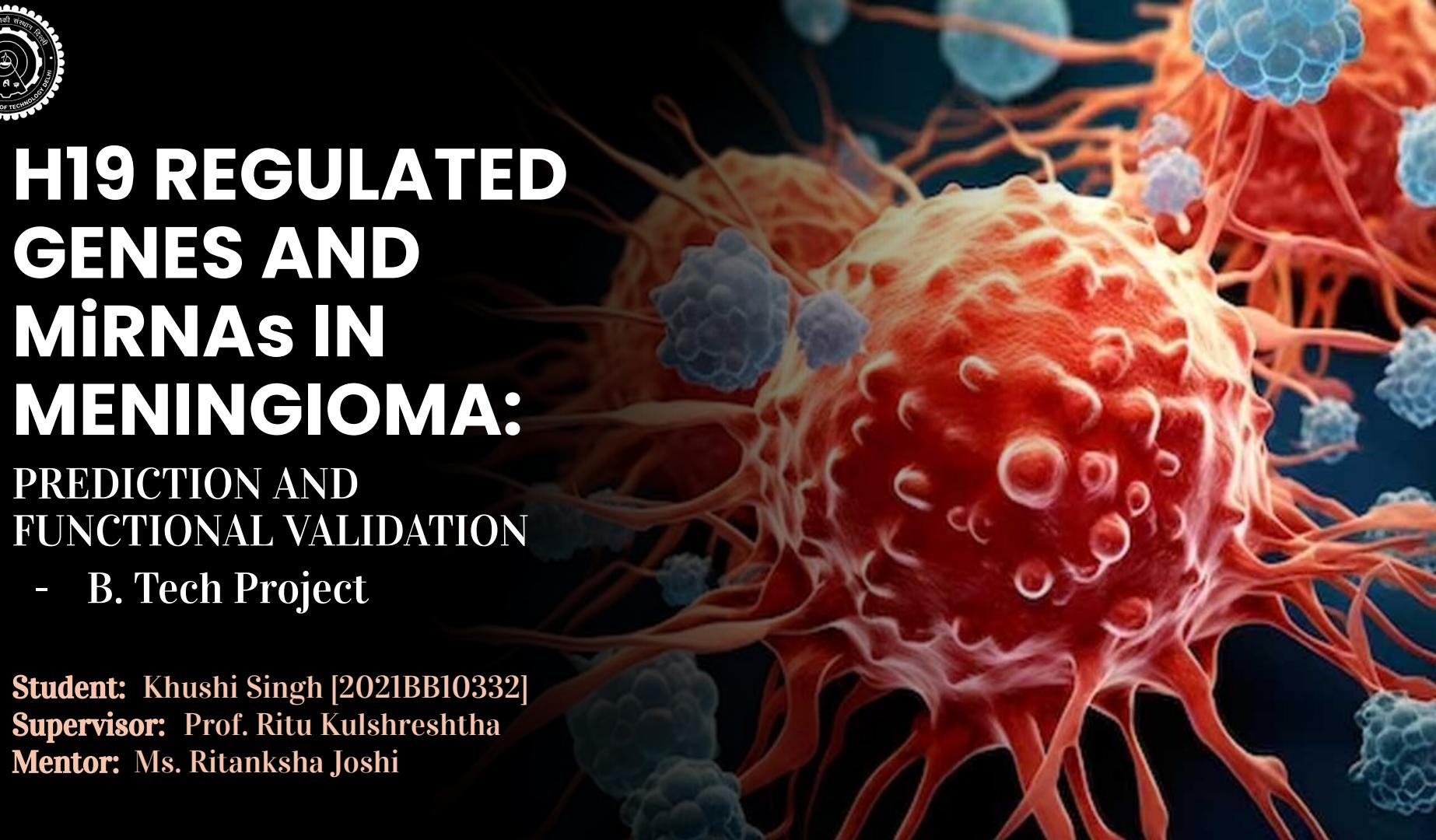
PREDICTION AND
FUNCTIONAL VALIDATION

- B. Tech Project

Student: Khushi Singh [2021BB10332]

Supervisor: Prof. Ritu Kulshreshtha

Mentor: Ms. Ritanksha Joshi



MENINGIOMA

Most common primary CNS tumour (37.6%)

Arises from the **meninges** of the brain and spinal cord

International incidence: 1.8 - 13 / 100,000

Mean age of presentation: **66 years**

Female-to-Male ratio: **2 : 1**

Recurrent tumours: Can re-establish after a remission period

WHO CLASSIFICATION (2021)

GRADE 1 (80.8%)

Mitotic rate:
 $<4/10$ high power fields

No brain invasion

GRADE 2 (18.3%)

Mitotic rate:
 $4-19/10$ high power fields

Brain invasion

≥ 3 of necrosis,
sheet-like growth,
prominent nuclei,
increased cellularity
and high
nucleus/cytoplasm ratio

GRADE 3 (1.6%)

Mitotic rate
 $>20/10$
high power fields

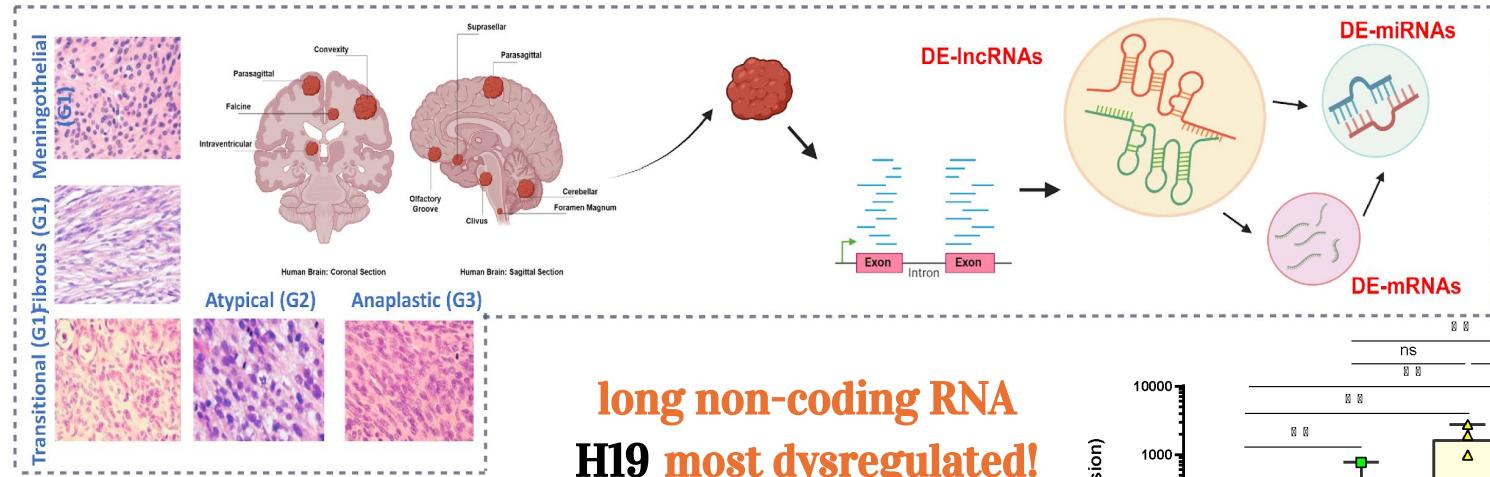
Brain invasion

TERT promoter
region mutations

CDKN2A/B
homozygous
deletion

BACKGROUND

A: Patient sample collection, histopathology & grading, RNA-Seq & Differential long non-coding RNAs (DE-IncRNAs) identified



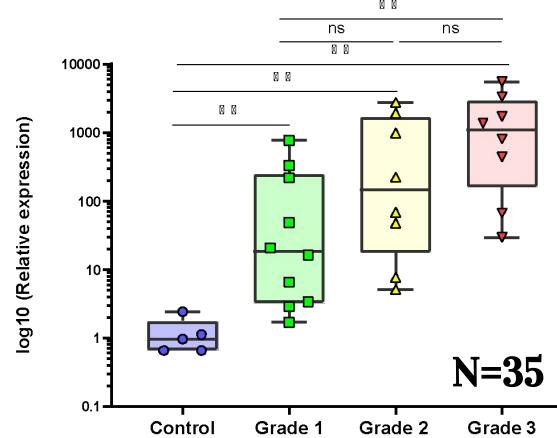
N=75

Sequencing Platform: Illumina HiSeqX Ten;
Depth: 50-80 million reads (150bp) paired end
Cutoff: | Log2FC | ≥ 2 AND P(adj) < 0.05

Credits: Ms. Ritanksha Joshi

long non-coding RNA
H19 most dysregulated!

Comparison	log2FC	p (adj)
G1 v C	6.31	7.66E-04
G2 v C	6.61	1.00E-04
G3 v C	8.21	7.67E-19



HYPOTHESIS

The long non-coding RNA **H19** plays a key role in
meningioma
pathogenesis via regulation of non-coding (miRNA) and
coding (mRNA) genes

NOVELTY

H19 is implicated in other cancers.
**NO STUDIES YET TO INVESTIGATE
H19's ROLE IN MENINGIOMA**

Normally H19 expressed exclusively from
maternal allele.

Loss of imprinting reported in meningioma.

OBJECTIVES

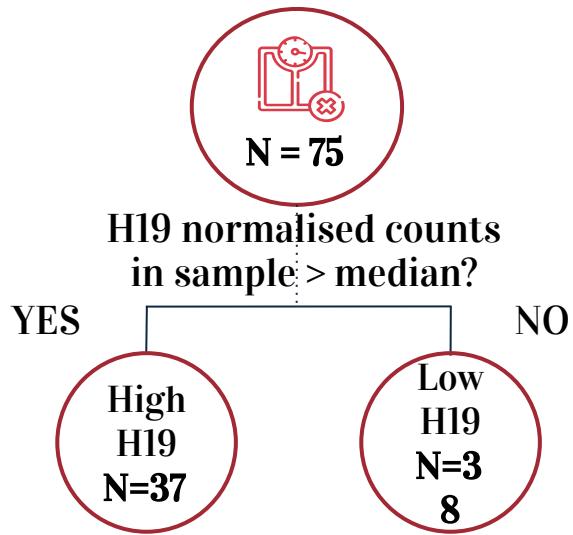
Derive
H19-ass
gene sign
menin

To study
associate
netwo
menin

Experi
validation
regul
genes/m
menin

Functional
validation of key
genes/miRNAs in
meningioma
through
cell-based assays

MATERIALS AND METHODS



Differential Gene Expression Analysis
of mRNA raw counts (R, DESeq2)
Cutoff: $|\log_{2}FC| > 1$ AND $padj < 0.05$

222
UPREGULATED

DOWNREGULATE
D

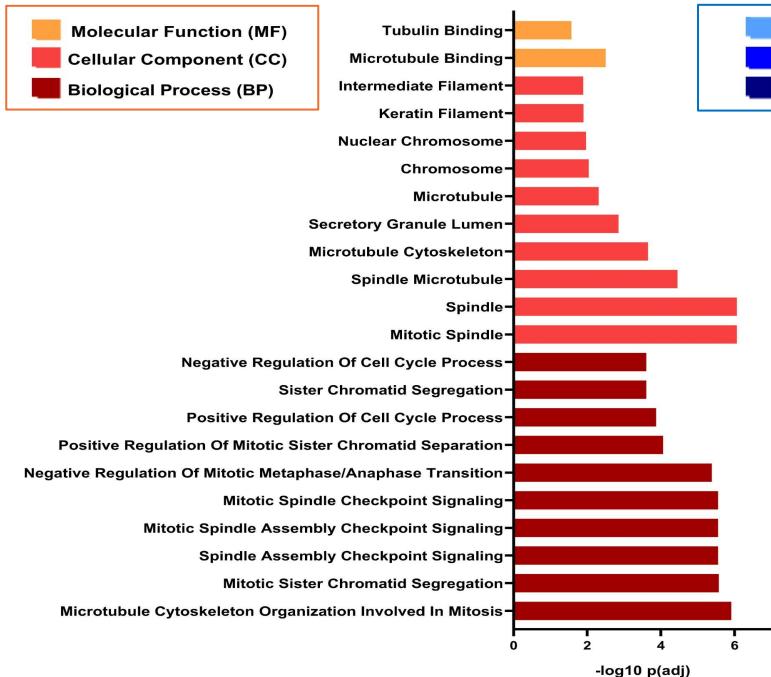
Sr. No.	Characteristic	Classification	Total Cases (n = 75)	High H19 (n = 37)	Low H19 (n = 38)
1	Age	< 60	63 (84%)	31 (49.2%)	32 (50.8%)
		> = 60	12 (16%)	6 (54.54%)	5 (45.45%)
2	Sex	Male	36 (48%)	20 (55.55%)	16 (44.44 %)
		Female	39 (52%)	17 (43.58%)	22 (56.41%)
3	Grade	Grade 1	34 (45.33%)	13 (38.23%)	21 (61.67%)
		Grade 2	35 (46.67%)	18 (51.43%)	17 (48.57%)
		Grade 3	6 (8%)	6 (100%)	0
4	Recurrence	Recurrent	21 (28%)	13 (61.9%)	8 (38.09%)
		Non-Recurrent	54 (72%)	24 (44.44%)	30 (55.55%)
5	Grade+ Recurrence	Grade 1 + Recurrent	11 (14.67%)	4 (36.36%)	7 (63.63%)
		Grade 1 + Non-Recurrent	23 (30.67%)	9 (39.13%)	14 (60.8%)
		Grade 2 + Recurrent	6 (8%)	5 (83.33%)	1 (16.66%)
		Grade 2 + Non-Recurrent	29 (38.67%)	13 (44.83%)	16 (55.17%)
		Grade 3 + Recurrent	4 (5.33%)	4 (100%)	0
		Grade 3 + Non-Recurrent	2 (2.67%)	2 (100%)	0
5	Subtype	Meningothelial (G1)	10 (13.33%)	6 (60%)	4 (40%)
		Transitional (G1)	16 (21.33%)	5 (31.25%)	11 (68.75%)
		Fibrous (G1)	3 (4%)	2 (66.66%)	1 (33.33%)
		Angiomatous (G1)	4 (5.33%)	0	4 (100%)
		Secretory (G1)	1 (1.33%)	0	1 (100%)
		Atypical (G2)	31 (41.33%)	18 (58.06%)	13 (41.93%)
		Clear Cell (G2)	2 (2.66%)	0	2 (100%)
		Chordoid (G2)	2 (2.66%)	0	2 (100%)
		Anaplastic (G3)	6 (8%)	6 (100%)	0
6	MIB LI	Low (< = 6)	39 (52%)	16 (41.02%)	23 (58.97%)
		Moderate (> 6, < 20)	26 (34.66%)	13 (50%)	13 (50%)
		High (>=20)	10 (13.33%)	8 (80%)	2 (20%)

DIFFERENTIALLY EXPRESSED GENES

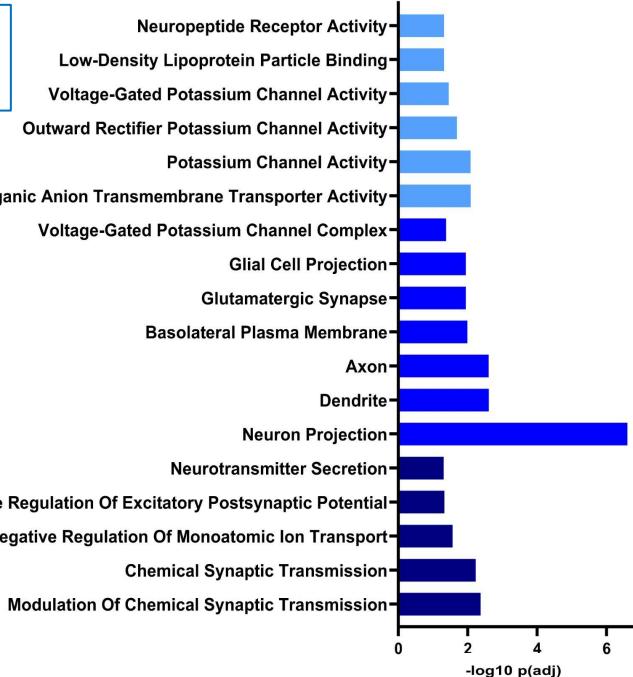
S. No.	Upregulated genes	log2FC	p (adj)	Downregulated genes	log2FC	p (adj)
1	AGTR2	3.18	2.65E-02	FUT9	-3.92	2.00E-05
2	POTEM	2.91	2.75E-02	ELMOD1	-3.36	4.60E-05
3	OR4M2	2.81	6.17E-03	CHL1	-3.14	1.34E-03
4	GPC3	2.80	1.54E-05	CDH18	-3.12	4.99E-03
5	MYF6	2.78	3.64E-03	SSTR1	-3.05	1.34E-03
6	KRT14	2.64	8.29E-03	RIMS2	-2.99	7.23E-03
7	PPP1R3A	2.54	1.12E-02	FRG2B	-2.86	1.27E-02
8	SLITRK6	2.52	2.19E-03	C11orf87	-2.85	3.22E-02
9	MYH2	2.44	2.18E-02	CLVS2	-2.78	3.57E-02
10	TDRD12	2.33	1.39E-03	ADCYAP1R1	-2.77	1.34E-03
11	IGF2BP1	2.30	2.07E-02	SLCO1A2	-2.75	1.92E-02
12	DMBT1	2.29	8.24E-03	NEGR1	-2.72	1.54E-05
13	TNNI1	2.28	2.87E-03	GPM6A	-2.67	3.38E-04
14	MYBPC2	2.27	4.69E-04	MCHR2	-2.66	4.18E-02
15	KRTAP10-2	2.26	3.87E-02	TRHDE	-2.65	4.22E-04
16	XIRP2	2.26	3.55E-02	FAM181B	-2.62	2.47E-03
17	SOX11	2.24	3.07E-03	AKRIB10	-2.61	1.34E-03
18	NMU	2.20	1.25E-02	APOD	-2.59	1.51E-03
19	MYH1	2.16	2.77E-02	PCDH11X	-2.55	1.80E-02
20	MMPI	2.16	3.89E-03	CNTNAP2	-2.55	1.69E-03

GENE ONTOLOGY ANALYSIS (Enrichr)

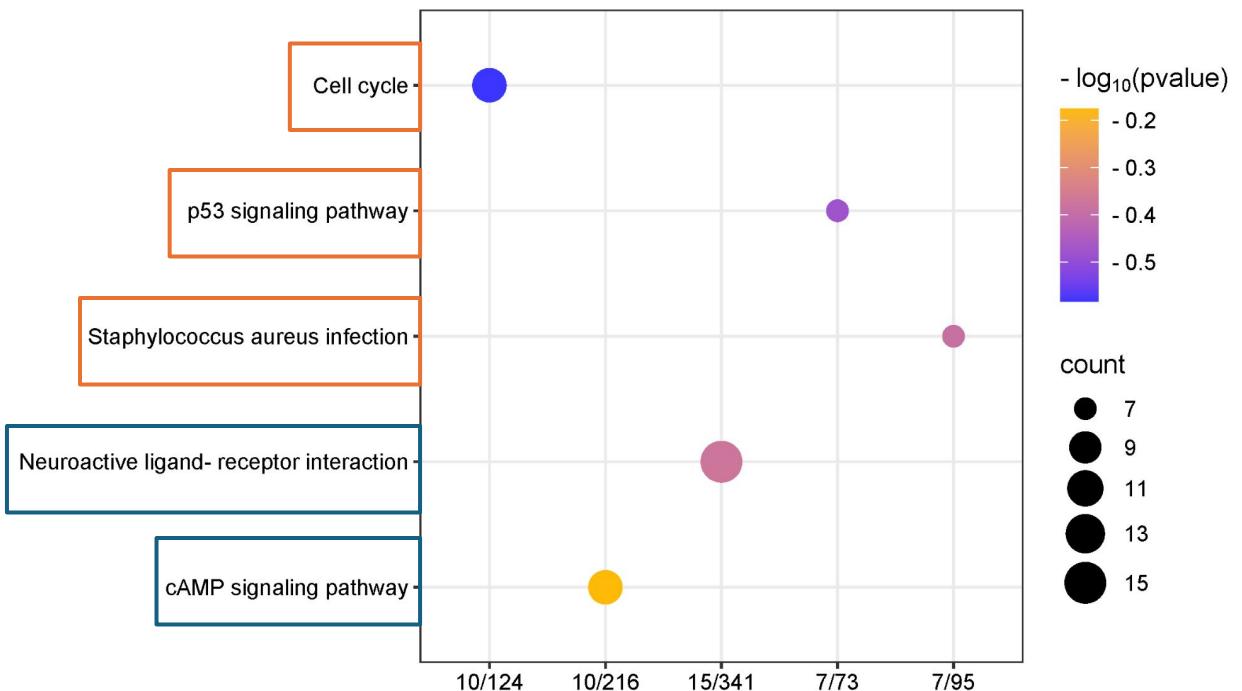
Upregulated DEGs



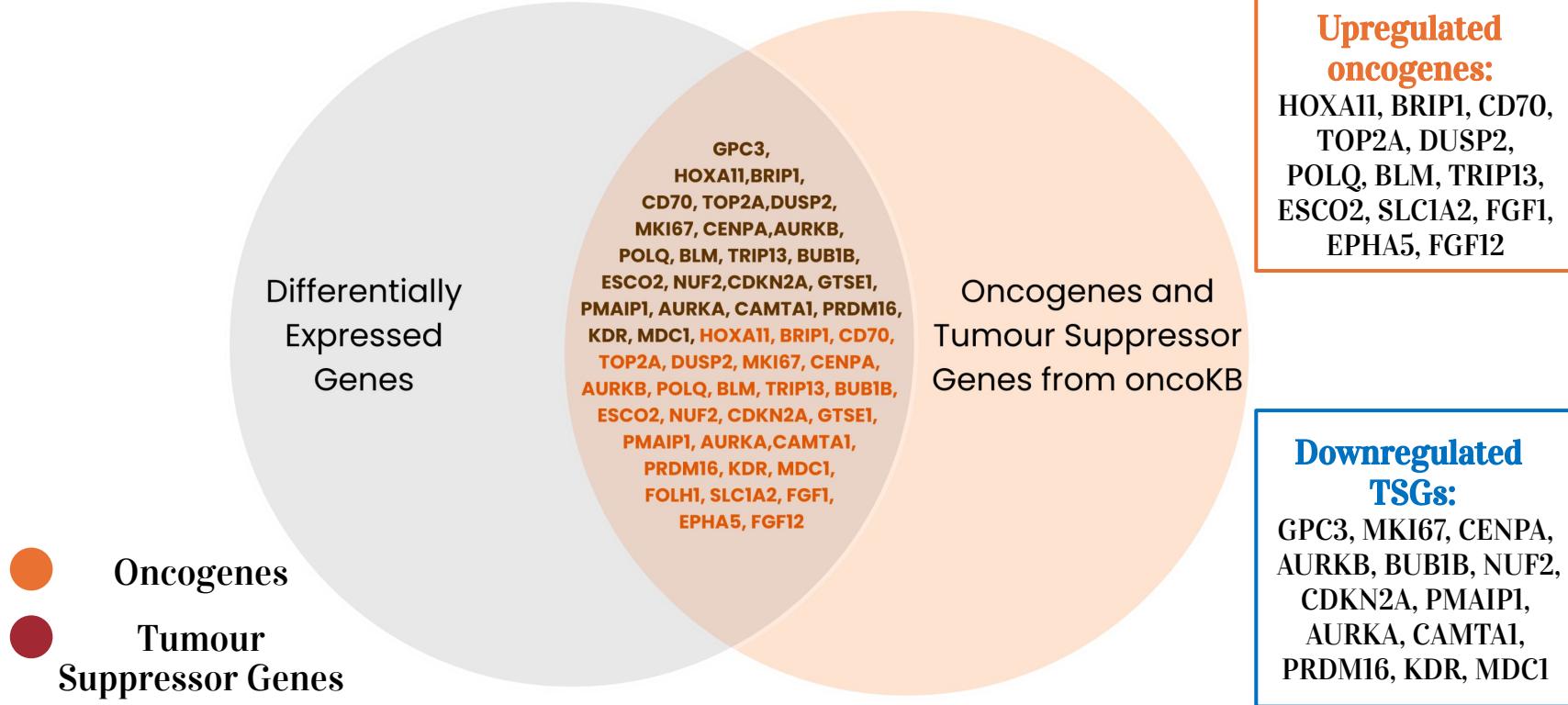
Downregulated DEGs



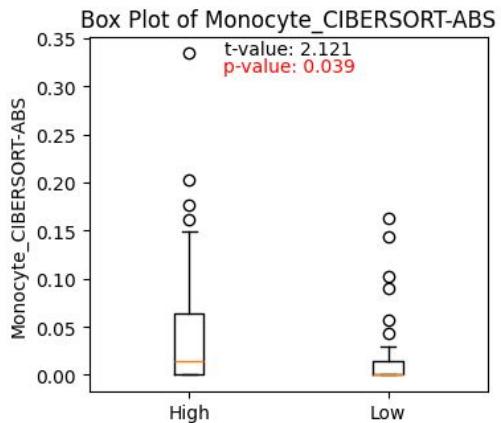
PATHWAY ANALYSIS (KEGG)



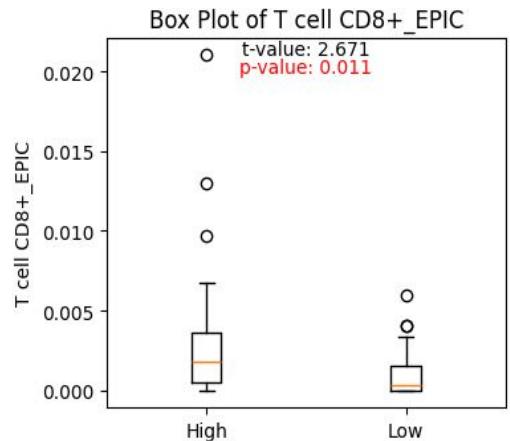
ONCOGENES/TSGs (OncoKB)



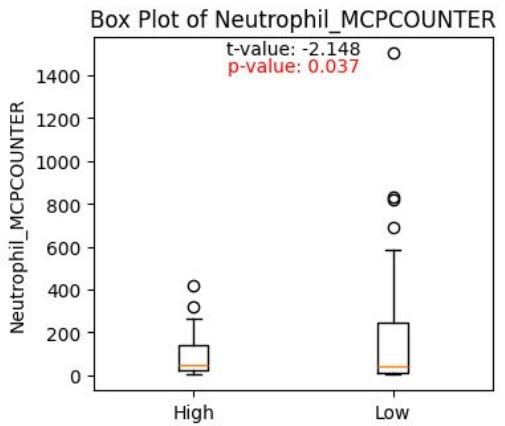
IMMUNE INFILTRATION (TIMER)



Increased in high H19



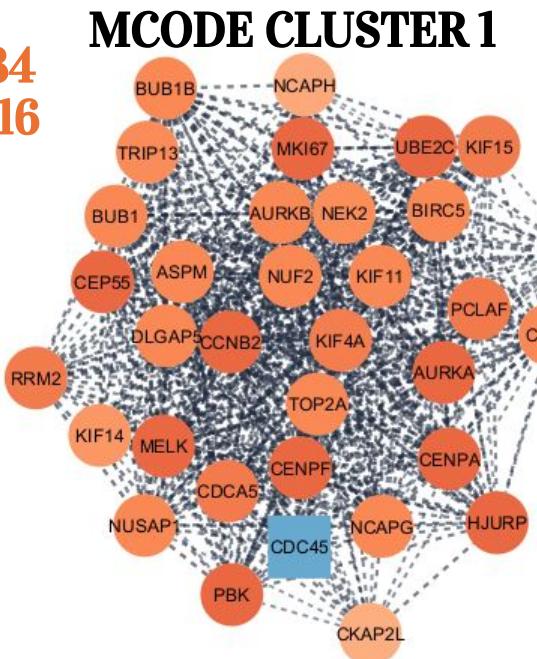
Increased in high H19



Decreased in high H19

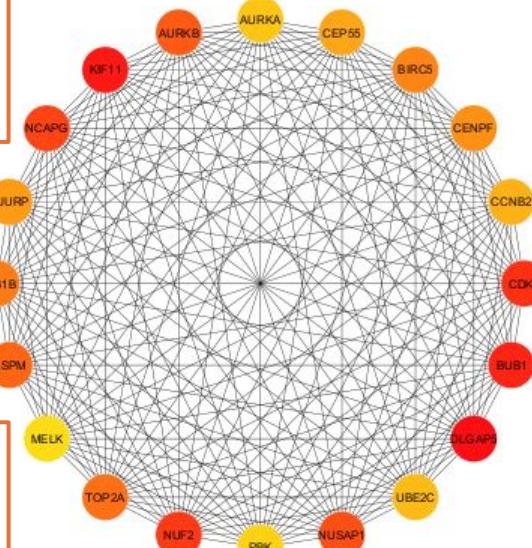
PPI NETWORKS Upregulated DEGs (STRING+CYTOSCAPE)

NODES: 34
EDGES: 516
SCORE:
31.273



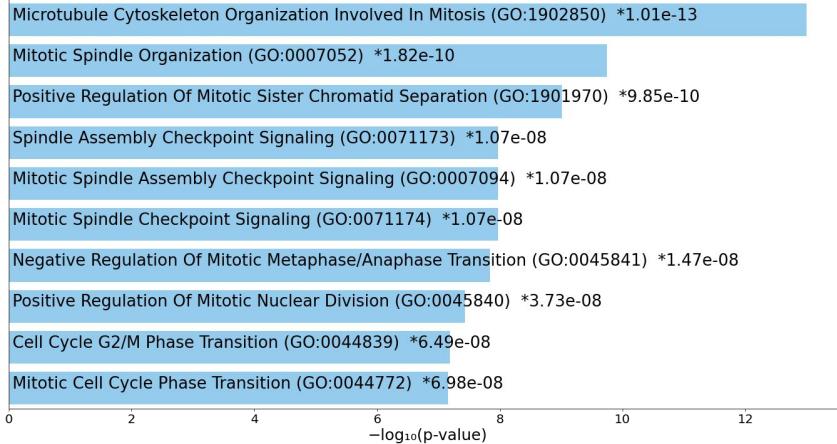
Progesterone
mediated
oocyte
maturation -
CCNB2, CDK1,
BUB1, AURKA

TOP 20 HUB GENES

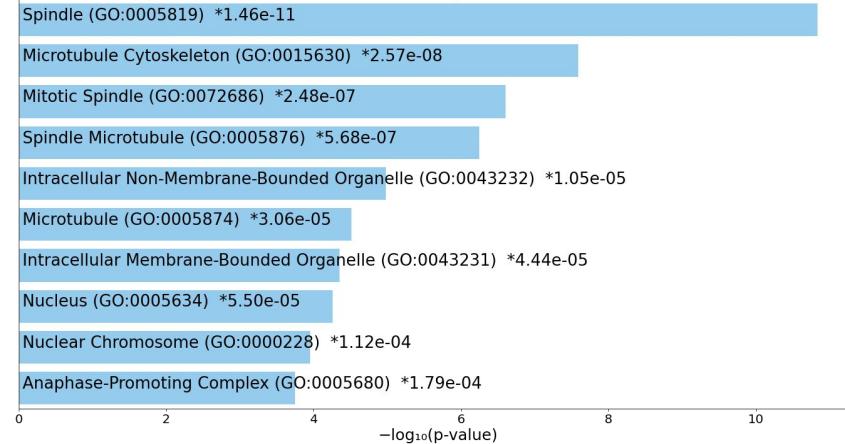


Cell cycle -
CCNB2, CDK1,
BUB1B, BUB1

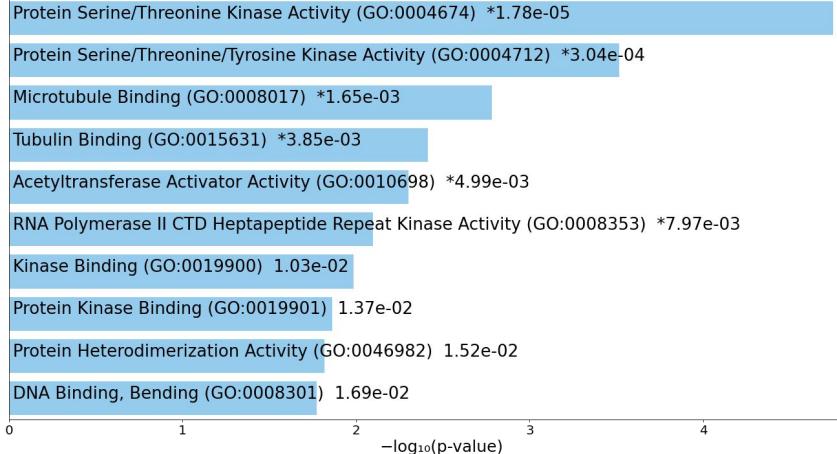
GO Biological Process 2023



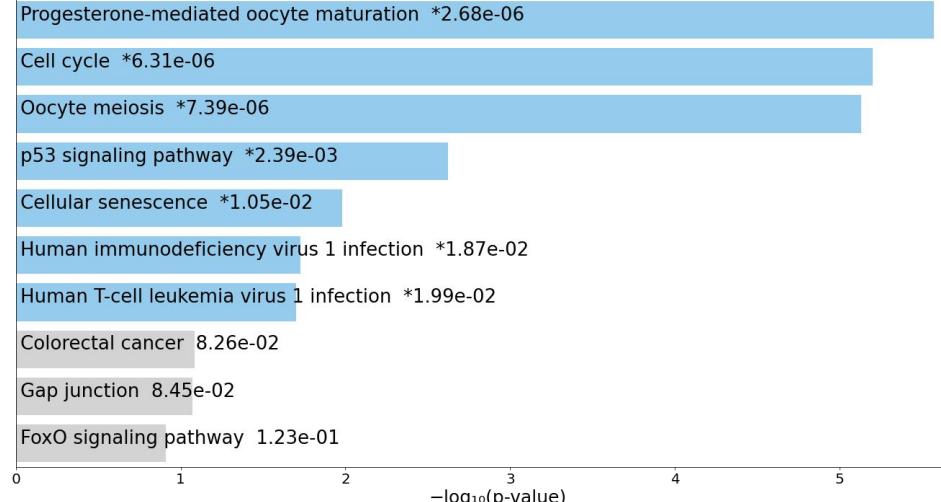
GO Cellular Component 2023



GO Molecular Function 2023



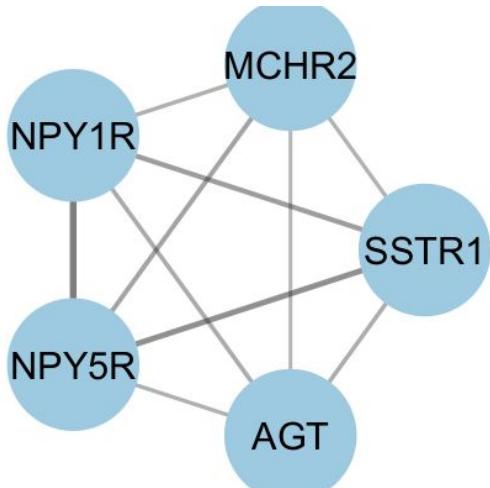
KEGG 2021 Human



PPI NETWORKS Downregulated DEGs (STRING+CYTOSCAPE)

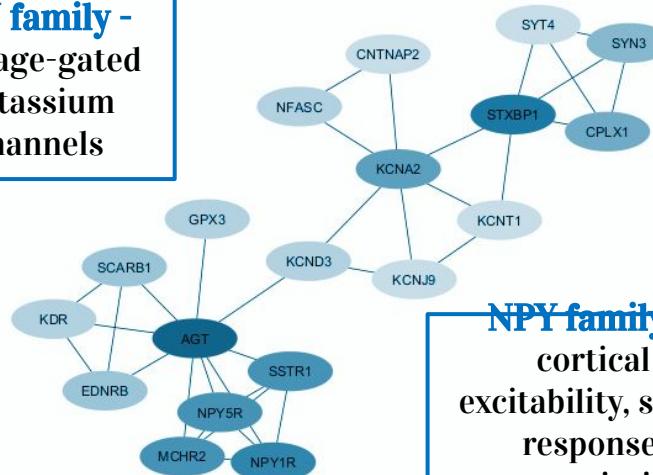
MCODE CLUSTER 1

NODES: 5
EDGES: 10
SCORE: 5



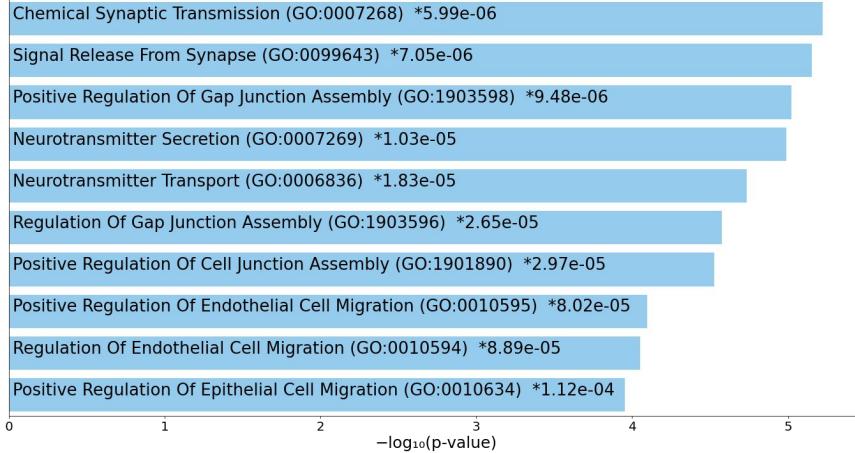
KCN family -
Voltage-gated
Potassium
channels

TOP 20 HUB GENES

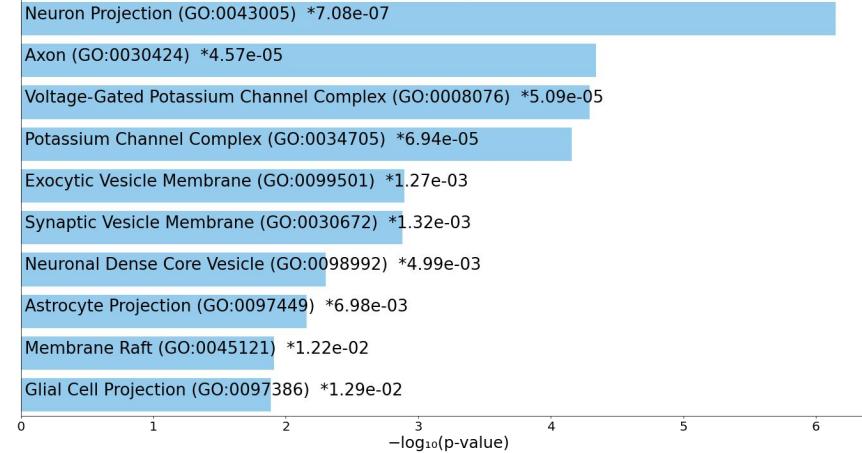


NPY family -
cortical
excitability, stress
response,
transcription,
GPCR downstream
signalling

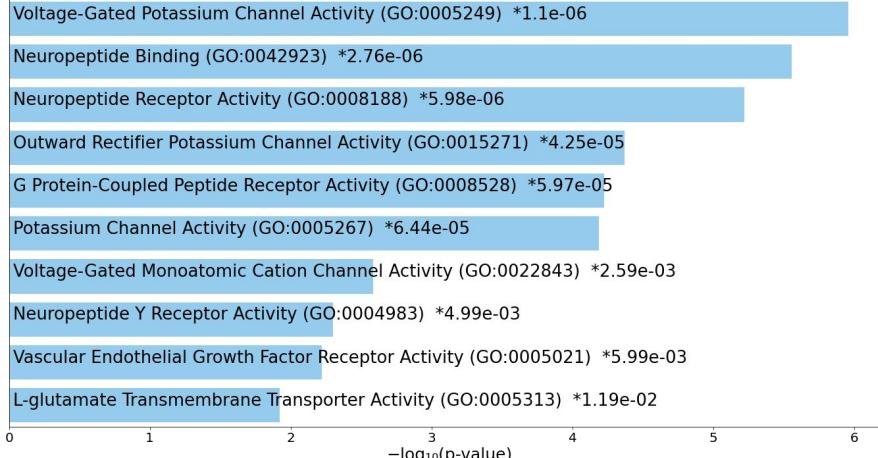
GO Biological Process 2023



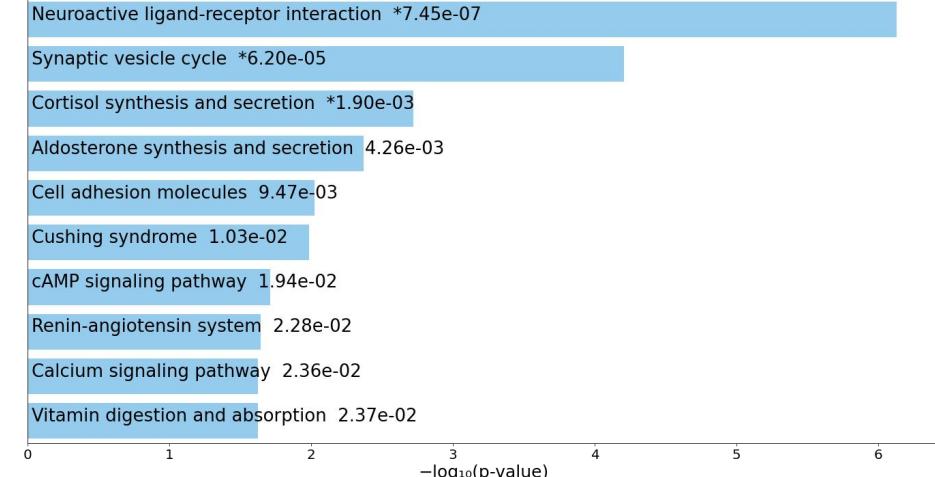
GO Cellular Component 2023



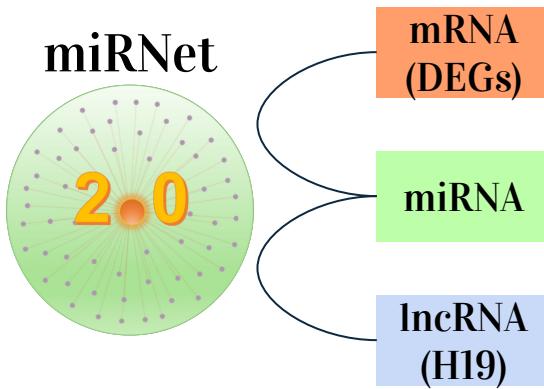
GO Molecular Function 2023



KEGG 2021 Human

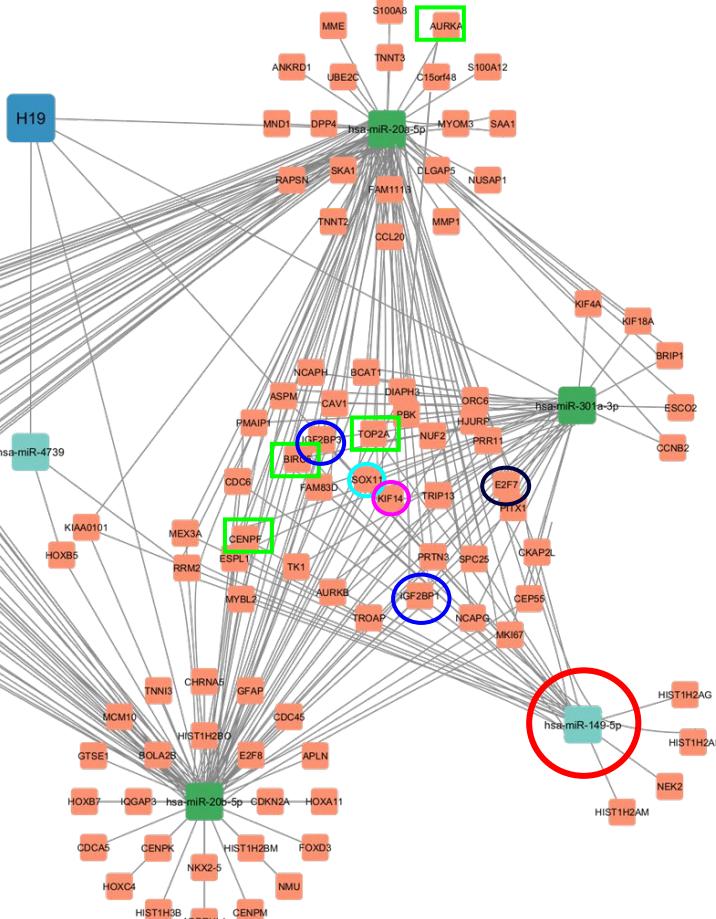


INTERACTION NETWORK



miRNA	Dysregulation	Implication in cancer studies
hsa-miR-20a-5p	Upregulated	Biomarker for cancer diagnosis and prognosis and therapeutic target in breast cancer, liver cancer, leukaemia etc. ²³
hsa-miR-20b-5p	Upregulated	Overexpressed in cancerous tissues, associated with lymph node metastasis, clinical stage, and overall survival, can promote malignancy of breast cancer cells and hepatocellular carcinoma cell proliferation, migration, and invasion ²⁴
hsa-miR-301a-3p	Upregulated	Promotes triple-negative breast cancer progression ²⁵ , inhibits killing effect of natural killer cell immunotherapy on non-small cell lung cancer cells ²⁶
hsa-miR-149-5p	Downregulated	Regulates inflammatory response, adipogenesis and cell proliferation, can function as an oncogene or tumour suppressor in different cancers ²⁷
hsa-mir-4739	Downregulated	Suppresses tumorigenesis and metastasis of hepatocellular carcinoma ²⁸ , suppresses the tumorigenesis and progression of prostate cancer ²⁹

miRNA	GenID	GeneName	GeneType	TargetSite	Alignment	Type	TDMDScore
hsa- miR- 149- 5p	ENSG00000130600	H19	↑	IncRNA↑	chr11:1997543- 1997563[-]	Target: 5' UGGAAAGAAG--GGGGAGCCAGG 3' ↑ : miRNA : 3' CCCUCACUUCUGUGCCUCGGUCU 5'	7mer-m8 1.2758 ↑

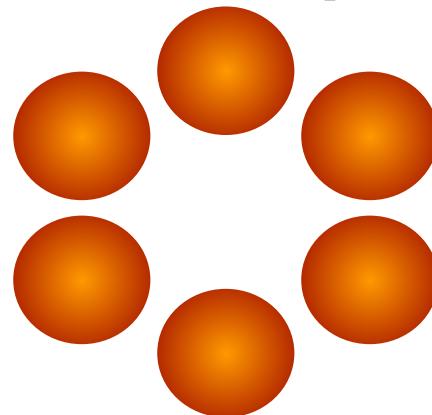


CONCLUSION

IDENTIFIED
TARGET MiRNA
hsa-miR-149-5p

222 upregulated
249 downregulated DEGs

GO and Pathway analysis:
Upregulated DEGs- Cell cycle
Downregulated DEGs-
neurotransmission



26 known onco
and TSGs

Prepared the
interaction network

Analysed immune
infiltration and PPI

FUTURE PLANS

Check **hsa-miR-149-5p** expression in IOMM Lee cells. (**RT-qPCR**)

Construction of
hsa-miR-149-5p
overexpression
construct (pcDNA 3.1+)

Validate H19 -
hsa-miR-149-5p
interaction
**(Dual-luciferase
Assay and/or RIP)**

H19 knockdown
(siRNA/shRNA),
check hsa-miR-149-5p
expression (**RT-qPCR**)

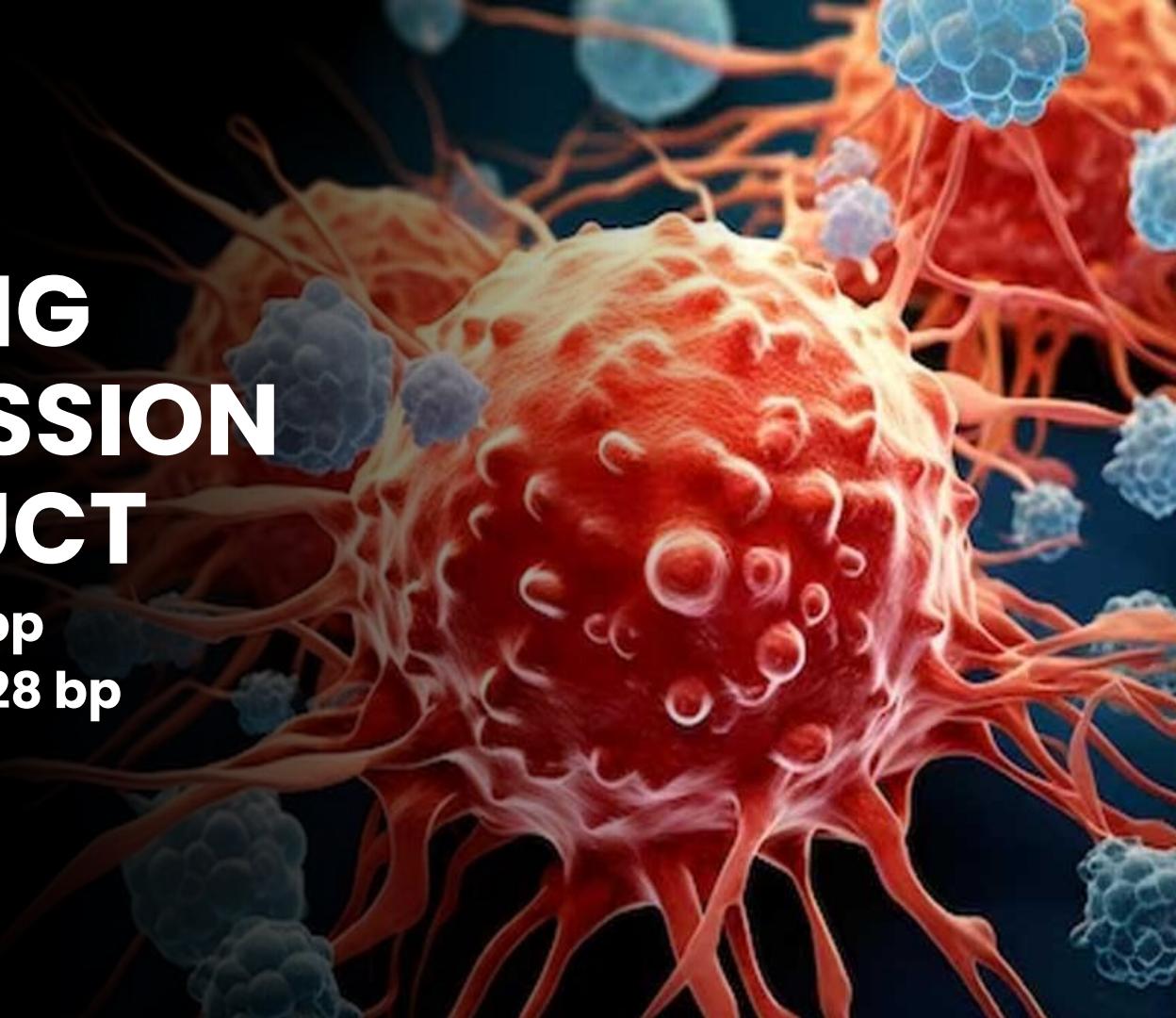
Construction of
luciferase reporter
construct with WT and
MUT H19 MRE sequence
(pMIR-REPORT)

Cell based assays to
functionally validate
miR-149-5p role in
meningioma
pathogenesis

CREATING OVEREXPRESSION CONSTRUCT

miR-149: 408 bp

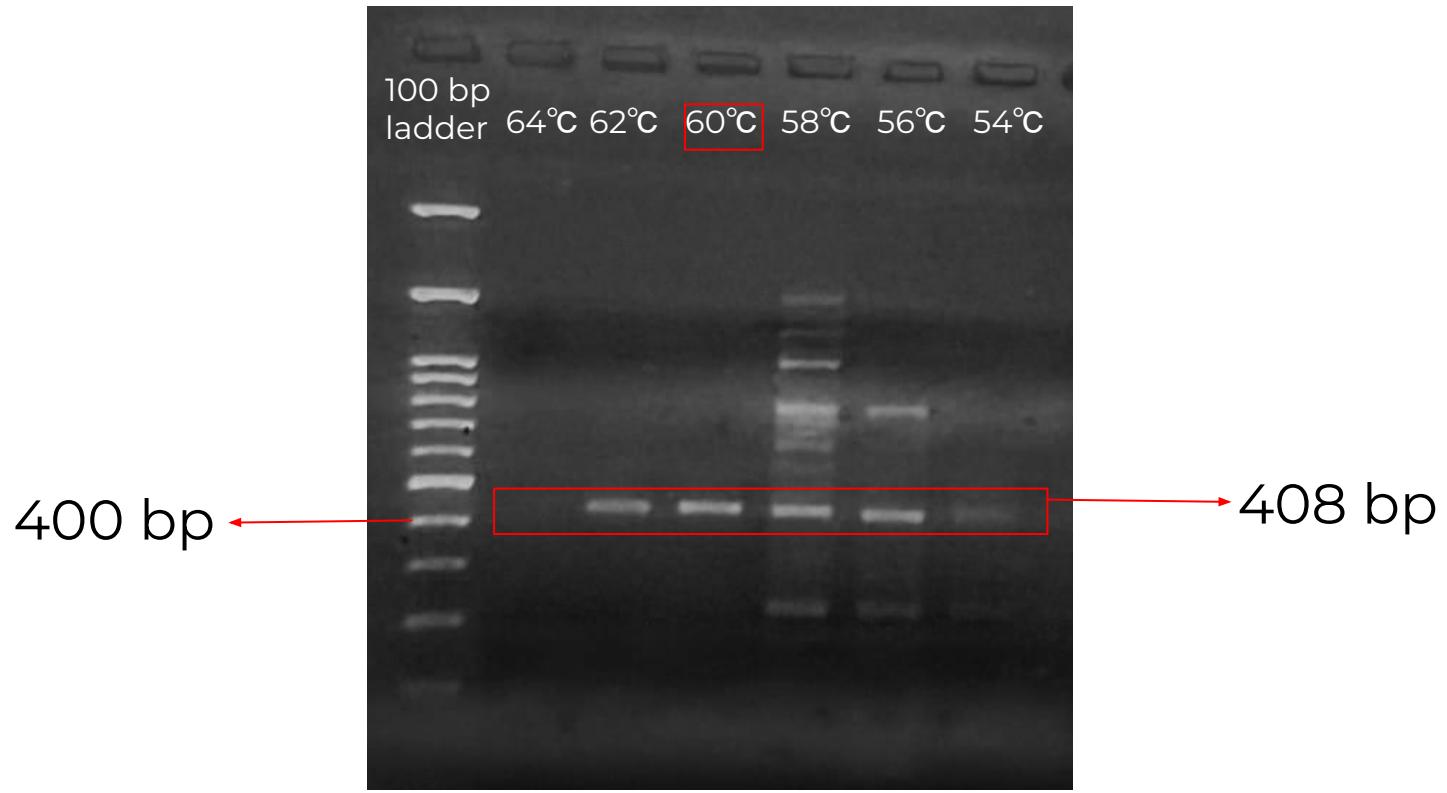
pcDNA 3.1 (+): 5428 bp



PRIMER DESIGN

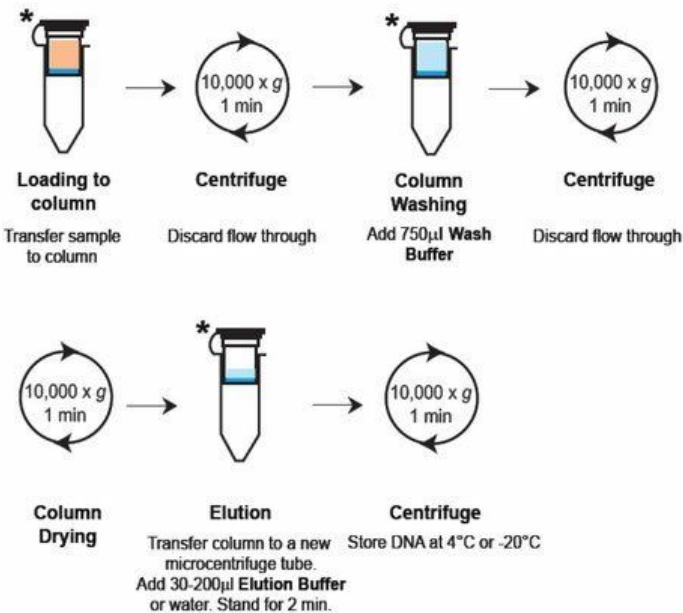
SR. NO.	NAME	SEQUENCE (5'-3')
1	miR 149- FP-CP Forward Cloning Primer	5' ATCAGGATCCCGCAGAAGGAAGCCAG 3'
2	miR 149- RP-CP Reverse Cloning Primer	5' ATTAGAATTCCGTAAGATATGGGAGCTCC 3'
3	miR 149-Stem Loop-RT Stem Loop sequence for miR-149	5'GTCGTATCCAGTGCAGGTCCGAGGTATTGCAC TGATACGACGGGAGT 3'
4	miR 149- 5pFP- QPCR Forward Detection Primer	5' TAGCTCTGGCTCCGTGTCTTC 3'
5	UnivRev P-QPCR Universal Reverse Detection Primer	5' GTGCAGGGTCCGAGGTATT 3'

TEMPERATURE STANDARDISATION



GEL ELUTION

Using thermo scientific GeneJET
Gel Extraction Kit

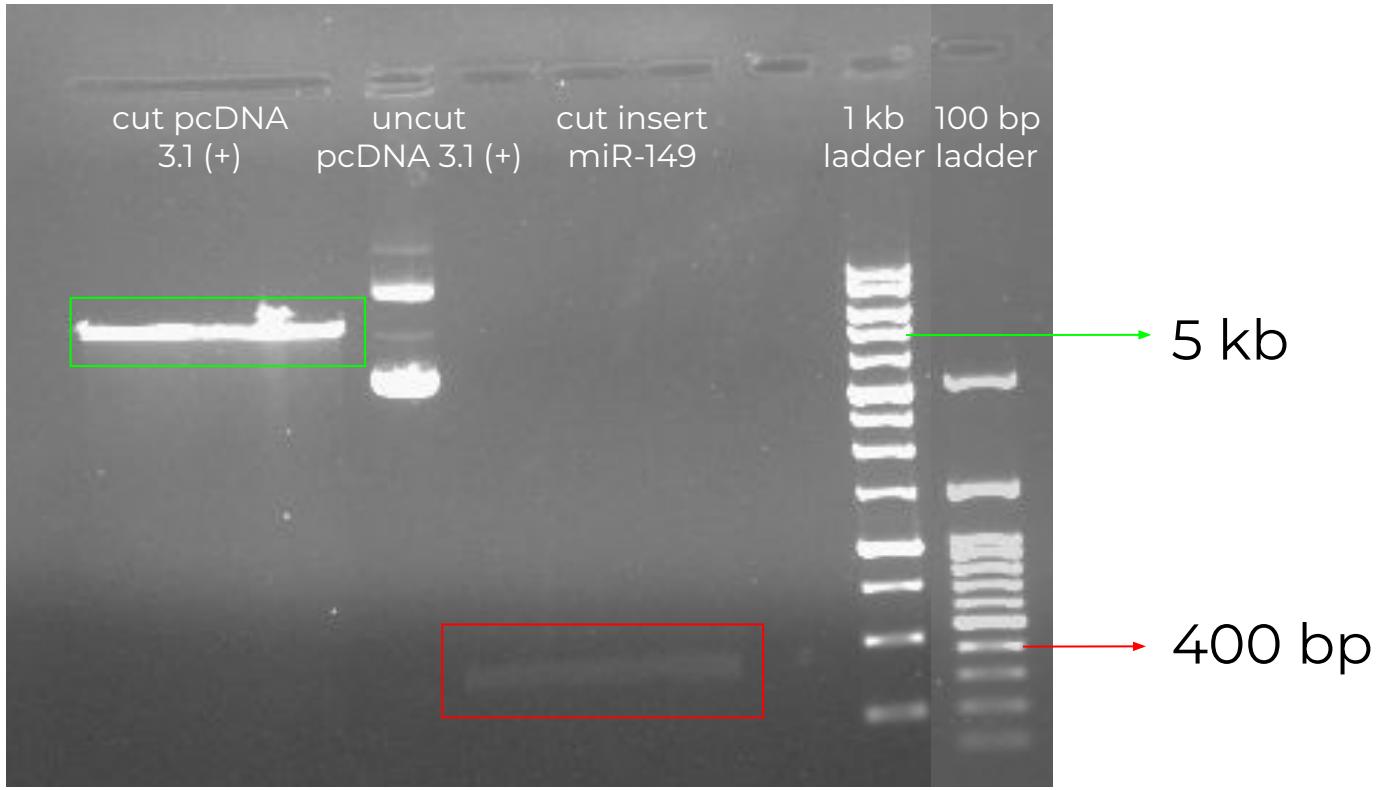


Nanodrop results:

Sample	Quantity (ng/µL)	A 260/280	A 260/230
I	16.9	2.05	0.02
II	13.9	2.11	0.02

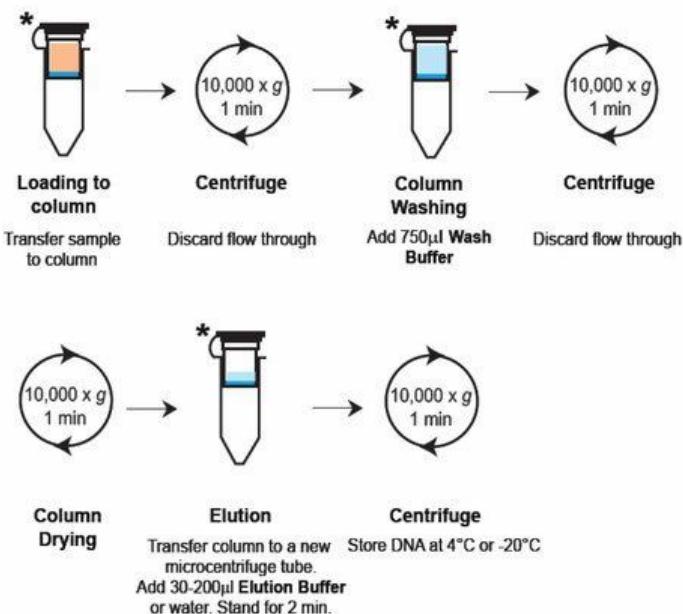
RESTRICTION ENZYME DIGESTION

- EcoRI
- BamHI



GEL ELUTION

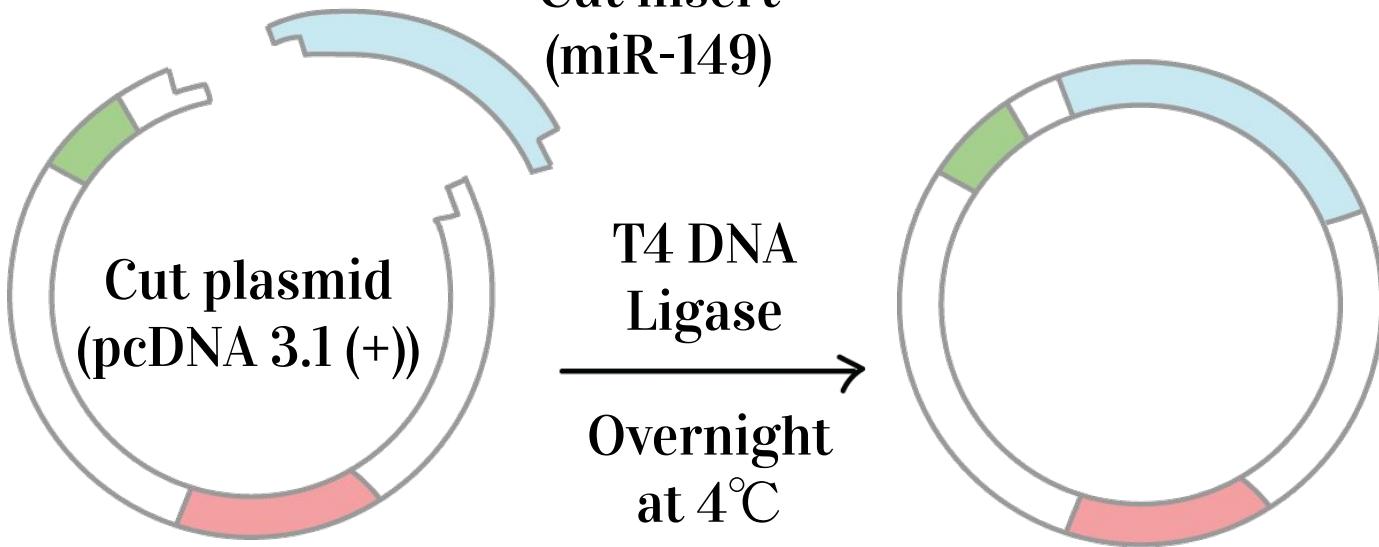
Using thermo scientific GeneJET
Gel Extraction Kit



Nanodrop results:

Sample	Quantity (ng/µL)	A 260/280	A 260/230
Plasmid	15.3	1.79	0.05
Insert	5.9	2.4	0.01

LIGATION



2 ligation reactions prepared - insert : vector ratio 5:1 and 7:1.

TRANSFORMATION

10 uL of each ligation mix + 50 uL of *E. coli* DH5 α competent cells
40uL/plate incubated O/N at 37°C

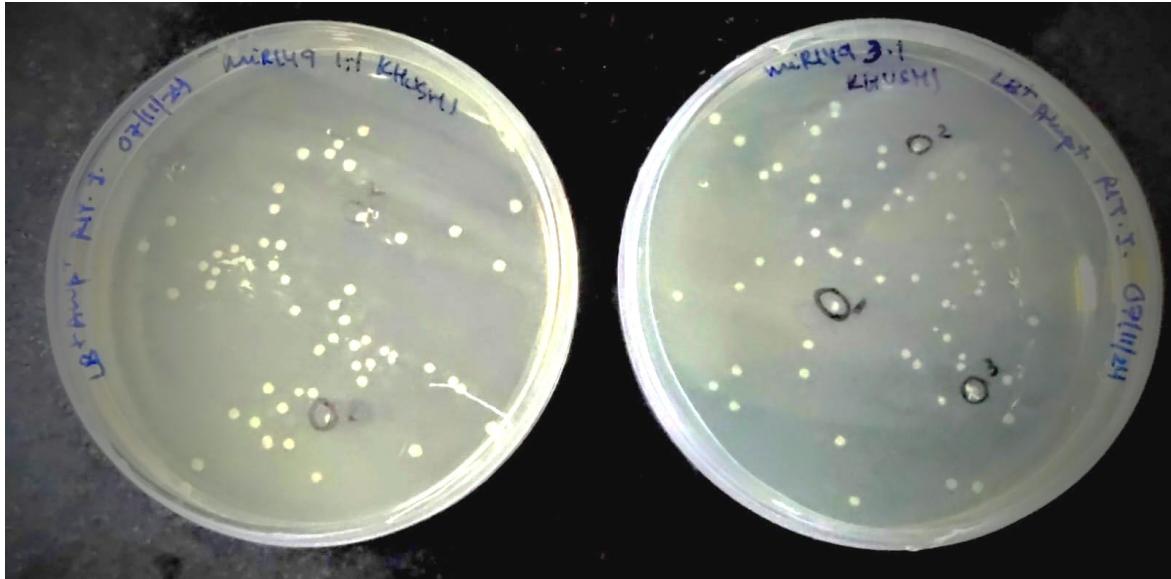
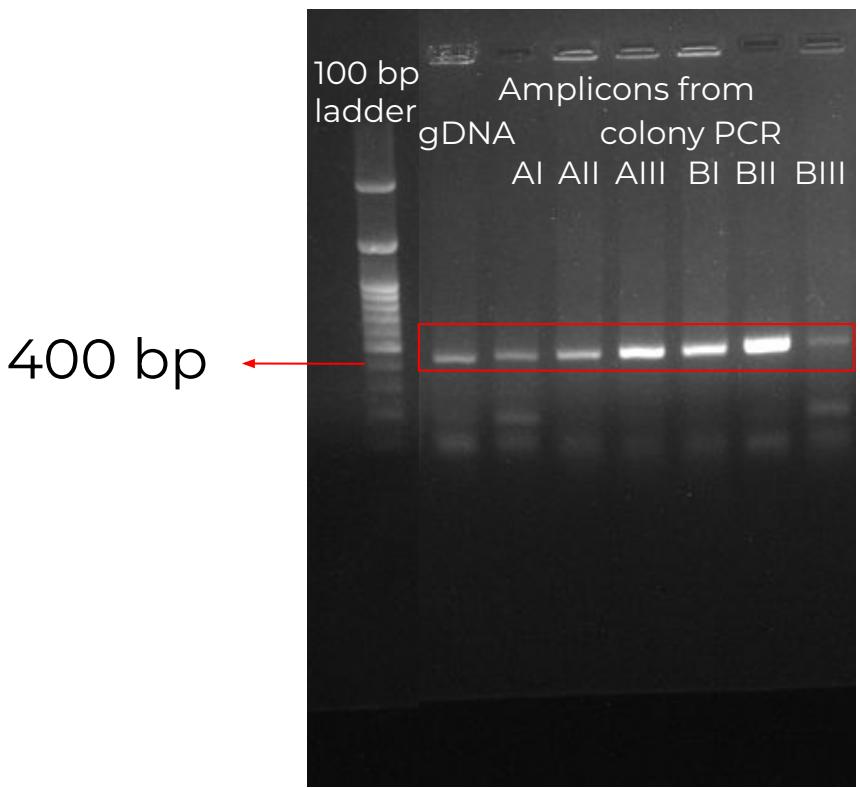


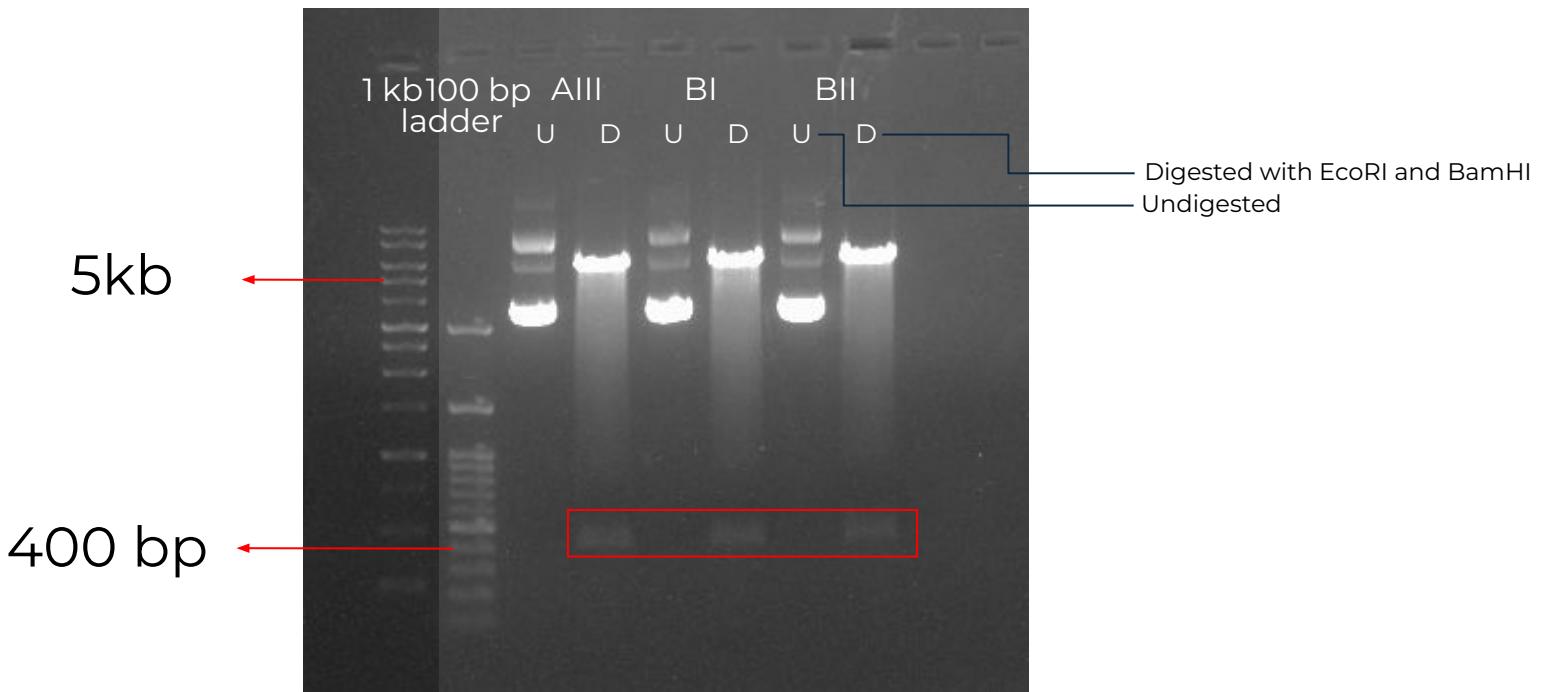
Plate A (5:1)

Plate B (7:1)

COLONY PCR



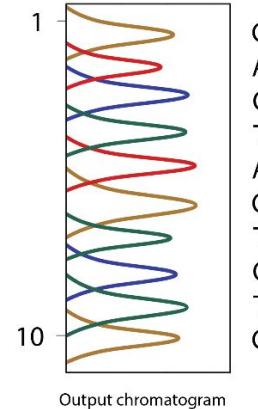
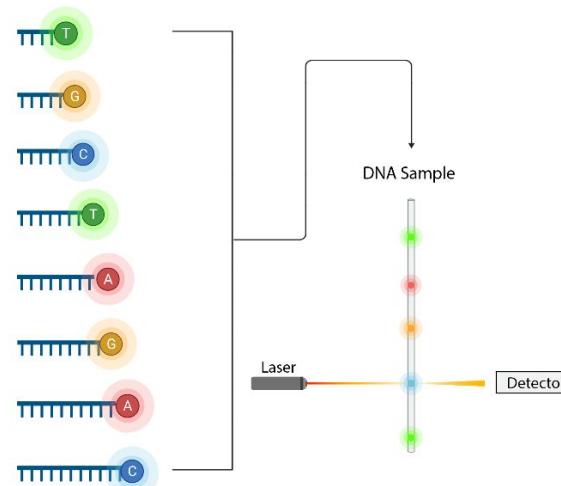
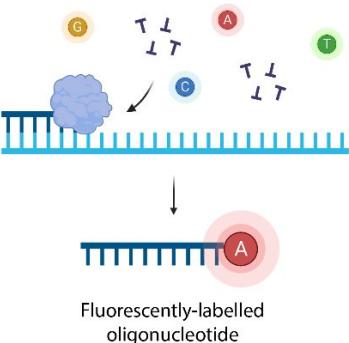
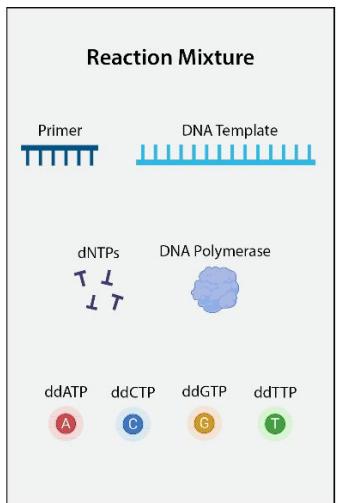
PLASMID EXTRACTION AND RE DIGESTION



SANGER SEQUENCING

The clones have been sent for Sanger Sequencing for final confirmation.

Sanger Sequencing



1

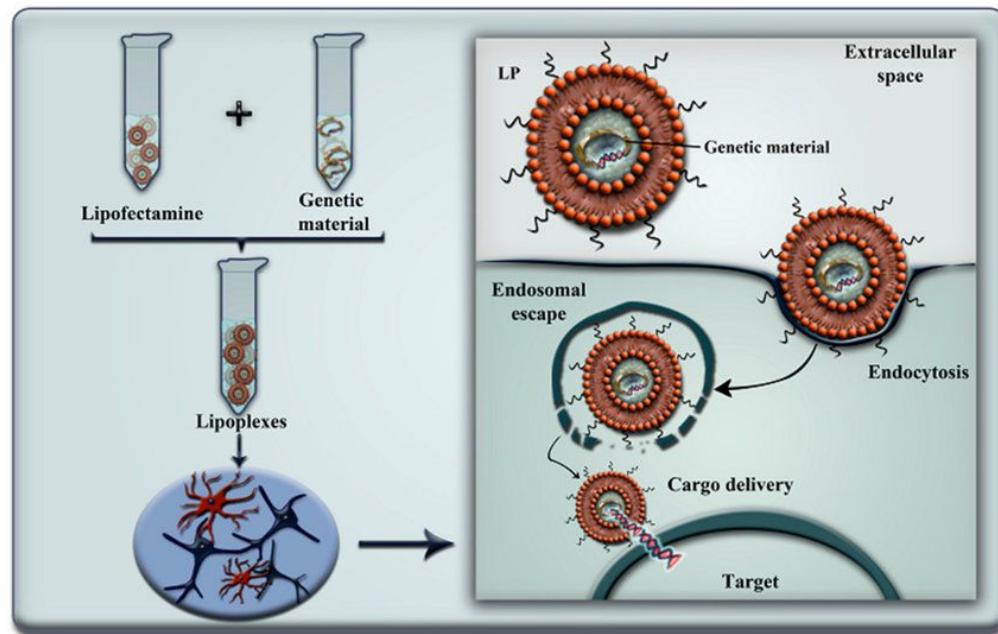
Chain-termination PCR using fluorescent ddNTPs

2

Size separation and sequence analysis using capillary gel electrophoresis and fluorescence detection

TRANSFECTION

- IOMM Lee cells seeded in 6 well plate
- Seeding density: 2 Lakh cells/well
- Transfection 24h post seeding
- 3 ug miR-149 clone and pcDNA empty vector (control) + 4ul Lipofectamine
- media - OPTI-MEM



SUMMARY

The lncRNA H19 was found overexpressed across all Meningioma grades w.r.t Control with increasing expression with grade.

miR-149 was found to bind to H19 at the site:
5' TGGAAAGAAGGGGGAGCCAGG 3' for 28/32 H19 transcripts on Ensembl (all except H19-211, 217, 221 and 223)

miR-149 was found downregulated in AIIMS patient (N=75) RNA sequencing data while H19 was found upregulated

SUMMARY

Hypothesis- possible ceRNA axis where H19 plays oncogenic role by suppressing miR-149 expression which may have tumour suppressive properties by regulating key mRNAs.

We created an overexpression clone of miR-149 to test this.

Further validation of miR-149 overexpression and cell-based assays will provide insights into the functional role of miR-149

Interaction between H19 and miR-149 may be validated by Dual luciferase assay and RNA Immunoprecipitation.

**THANK
YOU**

