“Prevention is better than cure” — exploration in cancer

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NARAYANA
MEDICAL COLLEGE

Education

Master of Science

July 2003 - June 2005

Jiwaji University

Molecular & Human Genetics

Gwalior (Madhya Pradesh), India

Doctoral research (Molecular & Human Genetics)

January 2006 - October 2012

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

Study on the transcription factor, BRN3A, in etiology of uterine cervix cancer

Varanasi (Uttar Pradesh), India

Postdoctoral Research Experience

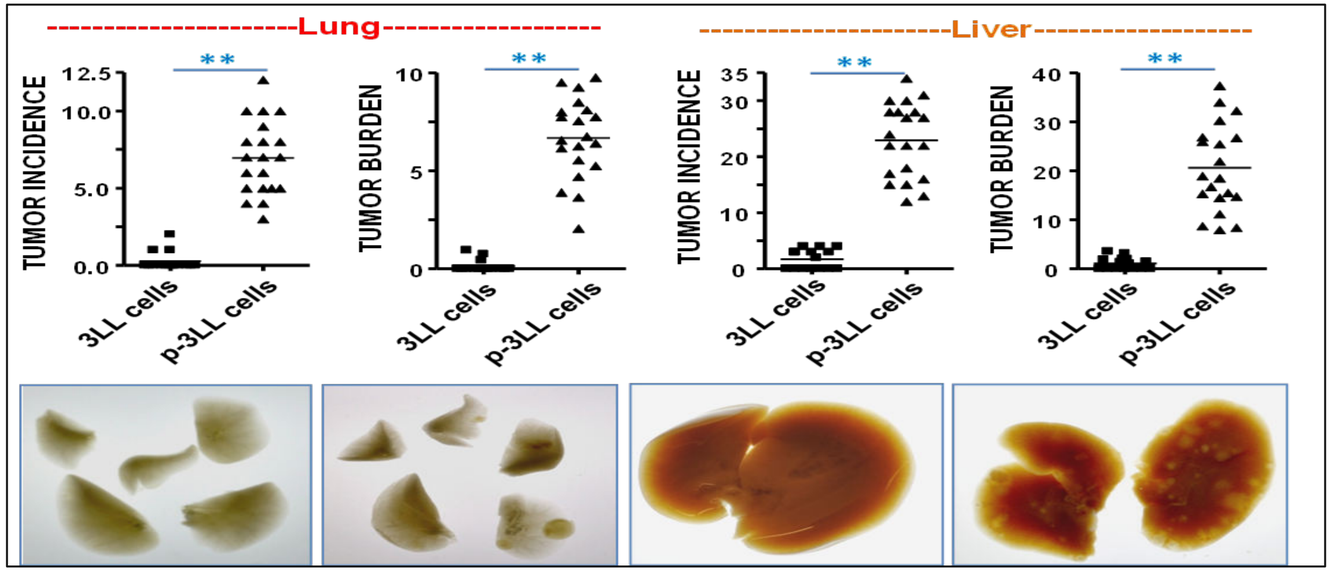
(Academic); March, 2015 - February, 2017

UNIVERSITY OF LOUISVILLE

Title: **Molecular mechanism of metastasis & chemoresistance in aggressive lung cancer**

Novel Research Finding/s:

- **Development of a robust lung cancer metastasis animal model**
- **Molecular mechanism of metastasis & chemoresistance in aggressive lung cancer**



Publication/s: (Total-2)

- A spontaneous metastasis model reveals the significance of claudin-9 overexpression in lung cancer metastasis, *Clinical & Experimental Metastasis*, 33(3), 263–275, 2016
- Hypertension exaggerates renovascular resistance via miR-122 associated stress response in aging, *Journal of Hypertension*, 36(11), 2226–2236, 2018

Postdoctoral Research Experience

(Academic); February 2017 - January, 2020

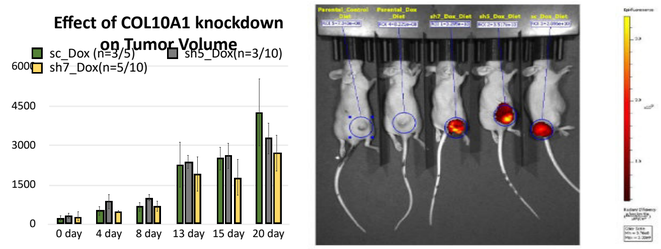
CASE WESTERN RESERVE UNIVERSITY EST. 1826

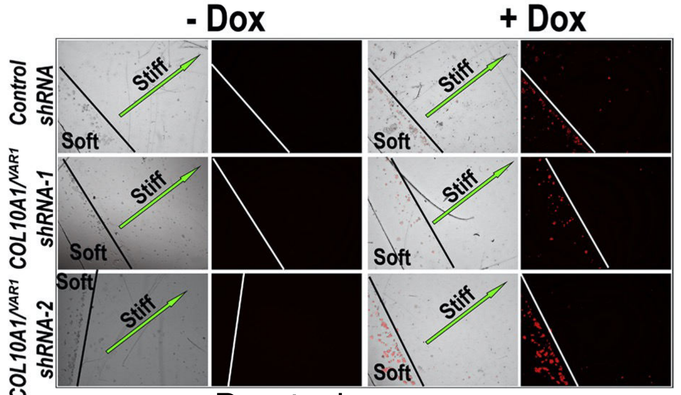
Title: **Understand the molecular genetics involved in the progression of Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC)**

Novel Research Finding/s:

- **Discovery of a novel transcript variant (*COL10A1^{Var}*) of *COL10A1* gene, and its identification as molecular biomarker for esophageal adenocarcinoma (EAC)**

- **Discover the familial basis of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC)**





Durotaxis assay

Publication/s: (Total-2)

- Genome-scale analysis identifies novel transcript variants in esophageal adenocarcinoma, *Cellular & Molecular Gastroentrology & Hepatology*, 2020
- Familial basis of BE and EAC (***Manuscript under preparation***)

GenBank

Homo sapiens collagen type X alpha 1 chain (COL10A1) mRNA, complete cds, alternatively spliced

GenBank: MN308081.1

FASTA Graphics

Go to:

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LOCUS       MN308081              3444 bp    mRNA    linear   PRI 07-MAY-2020
DEFINITION  Homo sapiens collagen type X alpha 1 chain (COL10A1) mRNA, complete
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VERSION     MN308081.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
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REFERENCE   1  (bases 1 to 3444)
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Industrial research experience

January 2020 - May 2021

Premas Biotech

Gurgaon (HR), INDIA

Development of 3-D mammalian cell-culture model using a patented technology

Novel Research Finding/s:

- Worked as scientific strategist in development of a patented 3D Cell-culture technology
- Also, successfully performed animal trial of Covid-19 vaccine, which would be the first oral Covid-19 vaccine

Publication/s: (Total-3)

- A Baru, S Sharma, **BP Das Purkayastha**, *et.al.*, (**Under review**). AXTEX-4D™: A novel 3D *ex vivo* platform for preclinical investigations of immunotherapy agents
- A Baru, S Mazumdar, P Kundu, S Sharma, **BP Das Purkayastha**, *et.al.*, (**Under review**). Recapitulating tumor microenvironment using preclinical 3D tissueoids model for accelerating cancer research and drug screening
- S Mazumdar, R rastogi, A Sundale, K Arora, N M Arora, **BP Das Purkayastha**, *et.al.*, (**Under review**). PRAK-03202: A triple antigen VLP vacine candidate against SARS CoV-2

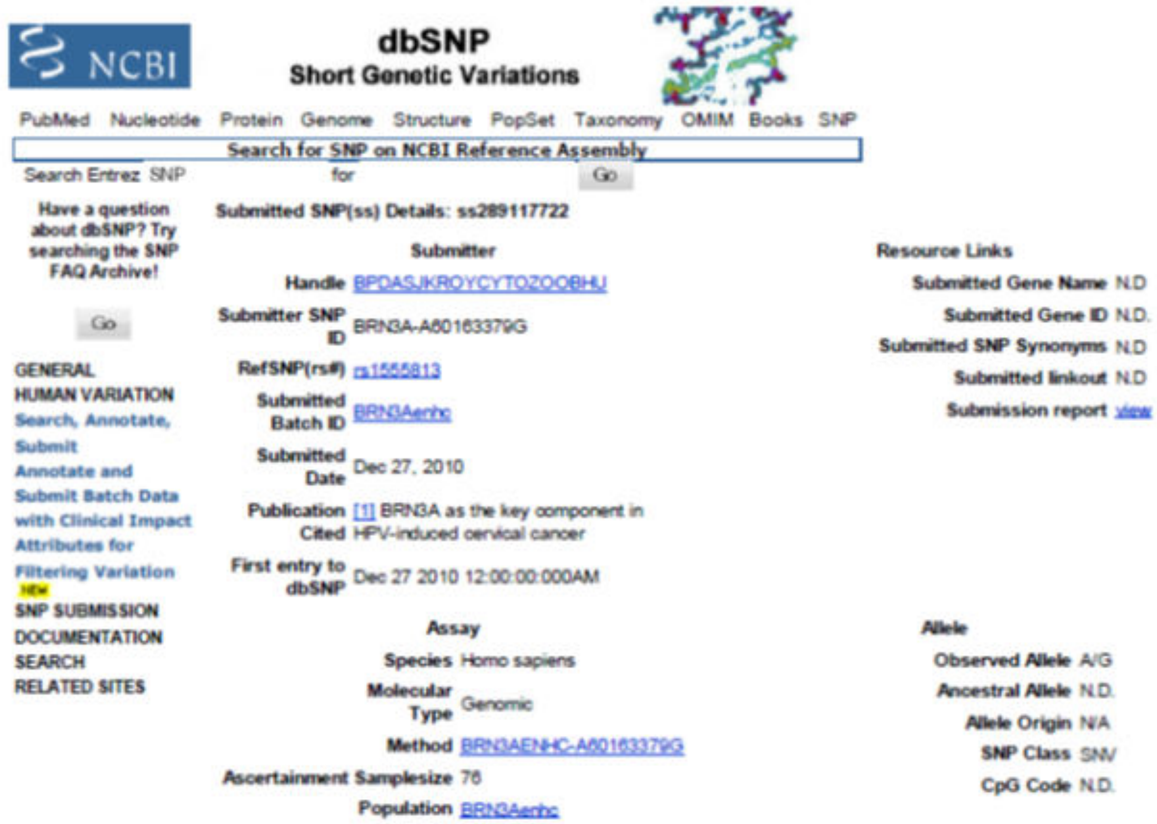
Doctor of Philosophy
(Molecular & Human Genetics)
January 2006 - October 2012

काशी हिन्दू विश्वविद्यालय
BANARAS HINDU UNIVERSITY

Title: **Study on the transcription factor, BRN3A, in etiology of uterine cervix cancer**

Novel Research Finding/s:

- Homeodomain transcription factor, BRN3A, is one of the key component in uterine cervix cancer and specifically in HPV-induced ones
- Discovery of a novel snp 60163379 A>G (Accession Number: ss289117722), which is modulating uterine cervix *BRN3A* expression in response to progesterone



NCBI

dbSNP
Short Genetic Variations

PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Books SNP

Search for SNP on NCBI Reference Assembly

Search Entrez SNP for Go

Have a question about dbSNP? Try searching the SNP FAQ Archive!

Go

GENERAL
HUMAN VARIATION
Search, Annotate, Submit
Annotate and Submit Batch Data with Clinical Impact Attributes for Filtering Variation NEW
SNP SUBMISSION
DOCUMENTATION
SEARCH
RELATED SITES

Submitted SNP(ss) Details: ss289117722

Submitter
Handle BPDASJKROYCYTOZOOBHJ
Submitter SNP ID BRN3A-A60163379G
RefSNP(rs#) rs1555813
Submitted Batch ID BRN3Aenhc
Submitted Date Dec 27, 2010
Publication Cited [1] BRN3A as the key component in HPV-induced cervical cancer
First entry to dbSNP Dec 27 2010 12:00:00:000AM

Assay
Species Homo sapiens
Molecular Type Genomic
Method BRN3AENHC-A60163379G
Ascertainment Samplesize 76
Population BRN3Aenhc

Resource Links
Submitted Gene Name N.D
Submitted Gene ID N.D.
Submitted SNP Synonyms N.D
Submitted linkout N.D
Submission report view

Allele
Observed Allele A/G
Ancestral Allele N.D.
Allele Origin N/A
SNP Class SNV
CpG Code N.D.

Prologue

- In a normal human body, ten million cells have been observed to be in the stage of division in every minute. Cell division occurs with utmost precision and may be accompanied by specialized development/differentiation

- Nevertheless, uncontrolled cell- division leads to the formation of mass of cells, which has been coined as 'tumour', and may finally culminate into one of the most deadly diseases haunting mankind, ‘malignant tumour’ or 'cancer'

- The term, ‘cancer’, was coined by the ‘Father of Medicine’, Hippocrates (400 BC), which originated from the term, ‘carcinos’ and ‘carcinoma’, a Latin word meaning ‘crab’.

NORMAL CELLS

CANCEROUS CELLS

Many cells that ontinue to grow and divide

Variations in size and shapes of cells

Nucleus that is larger and darker than normal

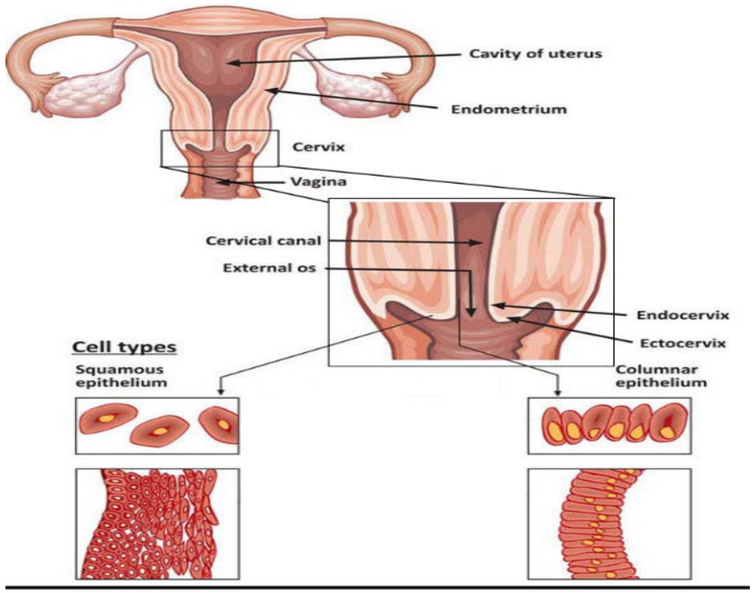
Abnormal number of chromosomes arranged in a disorganized fashion

Cluster of cells without a boundry

Study on the transcription factor, BRN3A, in etiology of uterine cervix cancer

Cervix (Latin *cervix uteri*, meaning "neck of the womb") is the narrow end of the uterus that opens into the upper part of the vagina and serves as the birth canal

Adapted from http://www.epgonline.org/

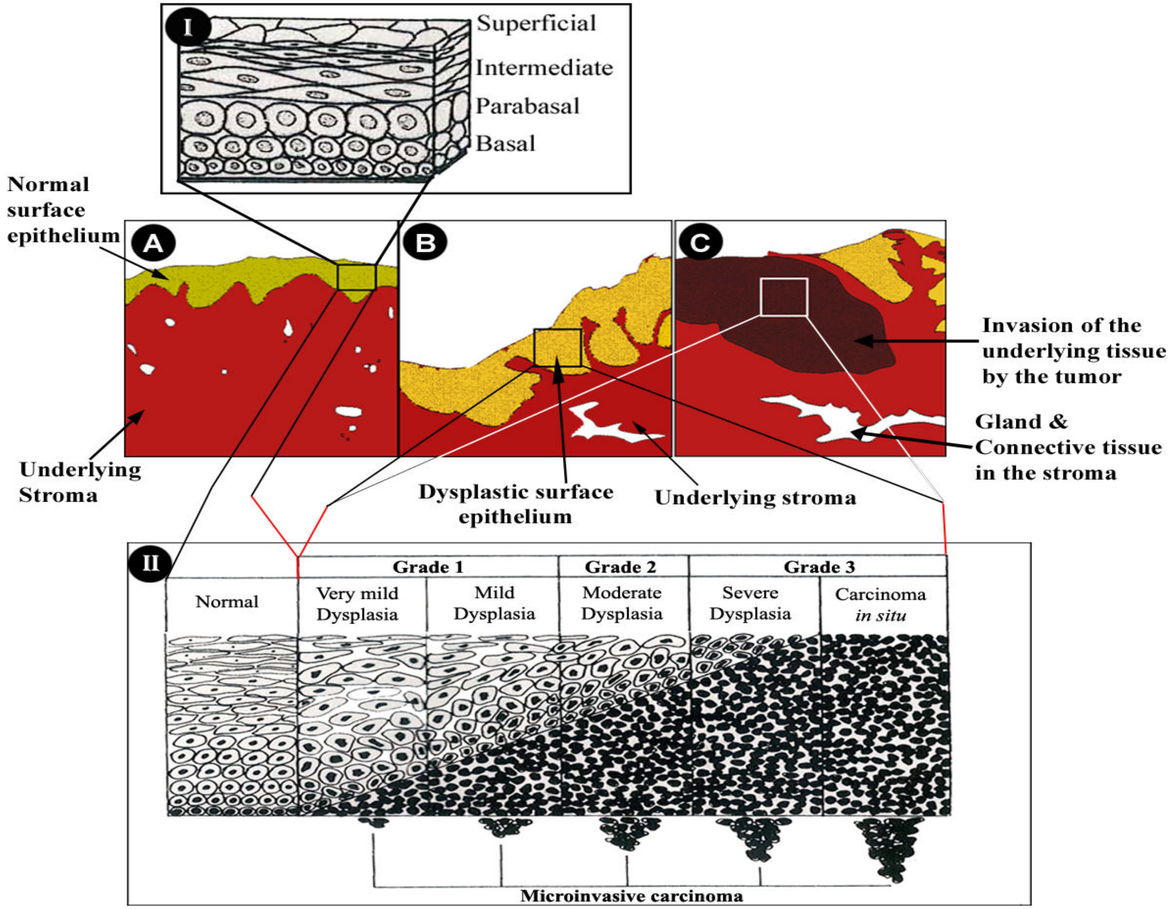


There are two epithelial linings in the uterine cervix

 # Squamous epithelium (Ectocervix)

 # Columnar epithelium (Endocervix)

The junction of these two epithelial linings, known as squamo-columnar junction, is the site of initiation of transformation and hence, this region is known as 'transformation zone'



Histology of uterine cervix. A) Normal epithelium **B)** Dysplastic epithelium, a potentially premalignant change **C)** Cancer originating from the surface epithelium and eventually invading the underlying stroma, which is abundant in connective tissue and endocervical glands **I)** Different types of cells present in the epithelium of the uterine cervix **II)** Representation of the spatial arrangement of the cells in different layers in the epithelia of the uterine cervix and its comparison with different grades of premalignant lesion. (Adapted and modified from: Hans-Friedrich, 2007 and Strachan and Read, 2011)

Risk factors associated with the development of the disease :

- Human papillomavirus (HPV) infection
- Sexual activity at an early age
- Multiple sexual partners
- High Parity
- Dietary factors and addiction
- Compromised immune system
- Other Sexually transmitted disease (STD)

HPV-TYPES

LOW RISK – (6, 11, 40, 42, 43 & 44)
INTERMEDIATE RISK – (31, 33, 39, 52 & 58)
HIGH RISK – (16, 18, 45 & 46)

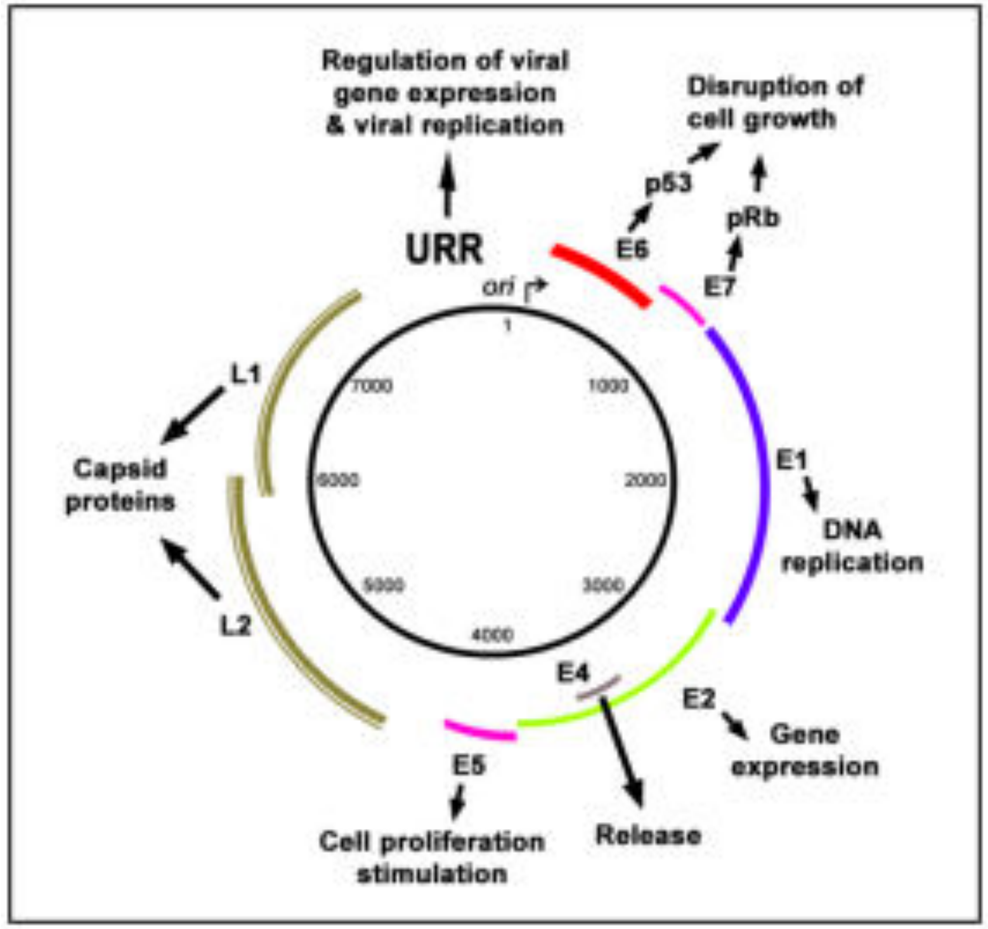
7.9% of women in the general population

~99% of invasive cervical cancers are positive for HPV

HPV contains a double stranded closed circular DNA genome of ~8kbp which is organized as –

EARLY genes
LATE genes

UPSTREAM REGULATORY REGION (URR)



- **Prof. Harald zur Hausen was awarded Nobel Prize (in Physiology or Medicine) in the year 2008 for the discovery that HPV is the causal agent for uterine cervix cancer**

- **Prof. Hausen also emphasized the role of cellular factors in the HPV-induced transformation process**

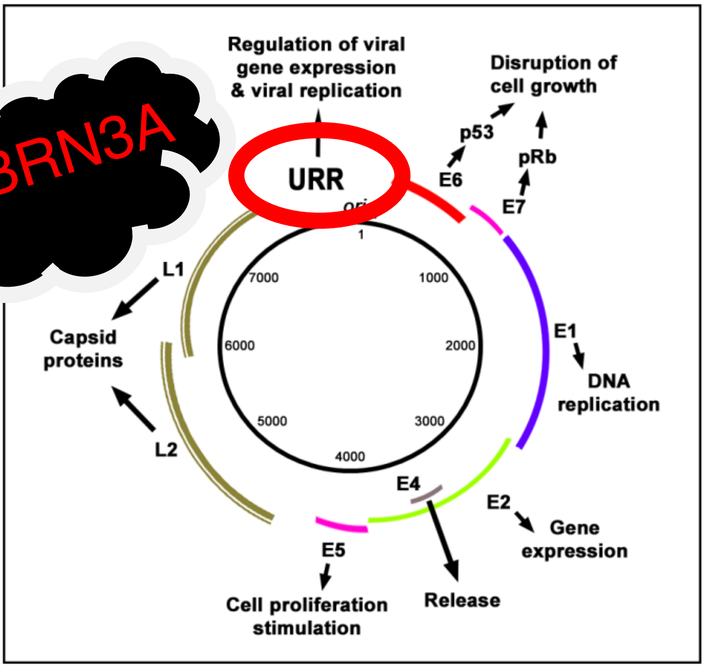
Albeit, human papillomavirus is widely known to be one of the major causes of the development of uterine cervix malignancy, the host plays potent role in the manifestation of the tumorigenic outgrowth.

This can be better exemplified by a very old hypothesis, dating back to 1967, embodied by Dr. Clyde E. Martin: “An infectious agent which is necessary although insufficient condition for the occurrence of the cancer”.

Why does the virus invade only the epithelia ?

The discovery of an octamer sequence – "ATGCAATT", in the region of the URR of HPV-16 and HPV-18 answered the question

- ❑ *BRN3A* silencing in HPV-positive cells, results in
  - ✓ Decreased cellular growth rate,
  - ✓ Reduced ability to form tumours and
  - ✓ (Most importantly) lowered the HPV-E6 expression
- ❑ BRN3A exhibits multiple folds overexpression in grade-3 of neoplasia as compared to that observed in the normal cervix



Introduction of BRN3A/ Brn3a

- Apart from its prominent role in cancer of the uterine cervix, BRN3A is also associated with other human disorders, like:
  - Neuroendocrine tumour
  - Autoimmune disorder
  - Acute myeloid leukemia and cancer of the prostate gland and ovary

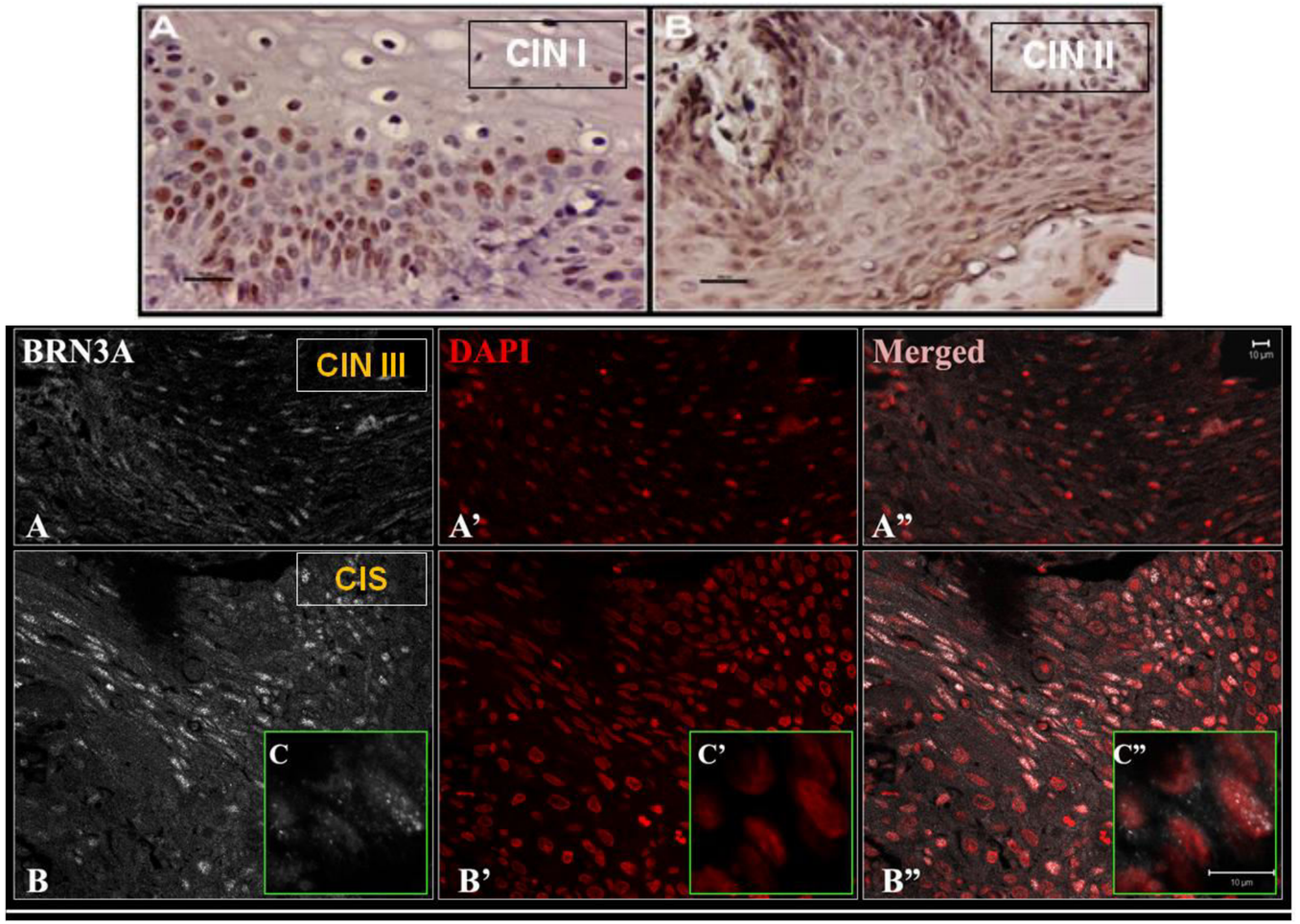
- Brn3a was initially identified to be a neuronal transcription factor, responsible for their survival and differentiation during the neuronal development
- Brn3a performs its anti-apoptotic functions in the neurons by regulating certain target genes like, Bcl-2, Bax, Noxa, etc.
- BRN3A is a homeodomain transcription factor. It is a member of the POU (Pit-1, Oct-1/2 & unc-86) family of transcription factors

Objectives of the study

- **Exploration of the transcription factor, BRN3A, expression and regulation in cancer of the uterine cervix**

- **Identification of the BRN3A interacting partners in cancer of uterine cervix in order to decipher the molecular mechanism exerted by BRN3A in uterine cervix tumorigenicity**

Differential expression of BRN3A in different stages of dysplasia of the uterine cervix

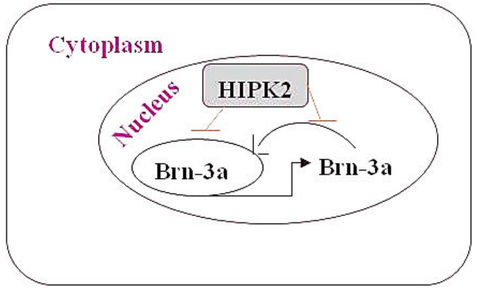


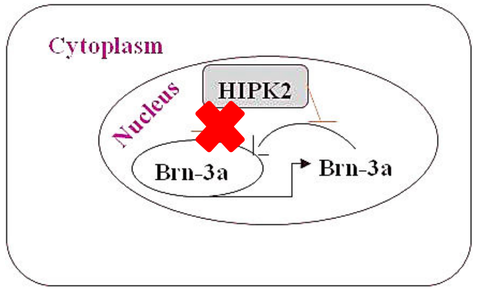
As dysplastic changes progress from the milder form to the severe ones extending to the carcinoma stage, the staining intensifies and the protein gets localized all over the cervix

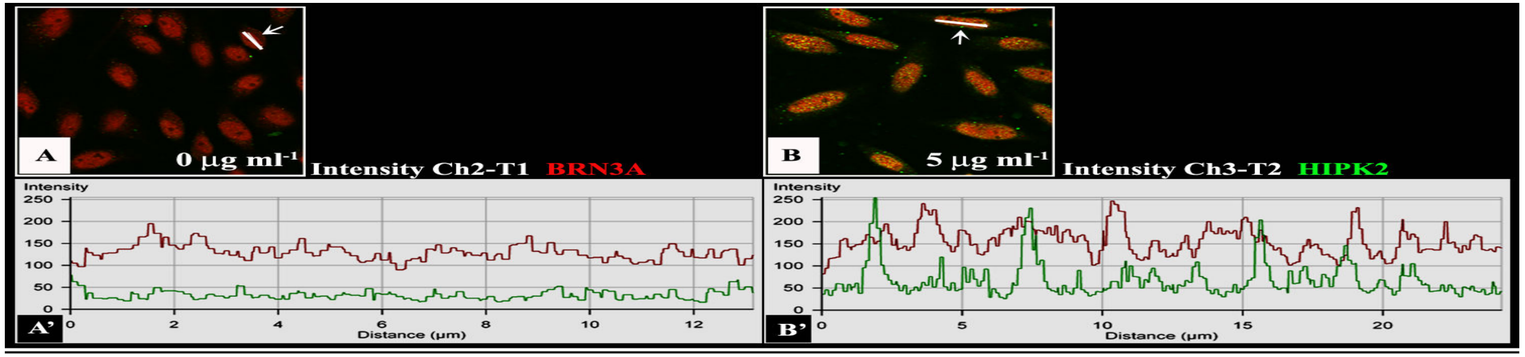
BRN3A expression directly correlates with the staging of the neoplastic growth in the uterine cervix and thus the expression possess histological significance

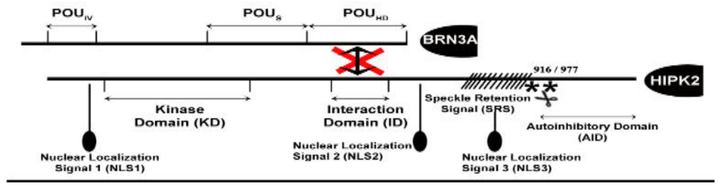
How is the oncoprotein, BRN3A, regulated in the uterine cervix?

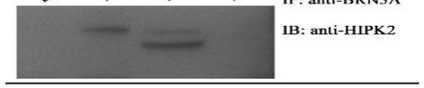
Elucidation of the pathway/s involved in the regulation of BRN3A in the uterine cervix

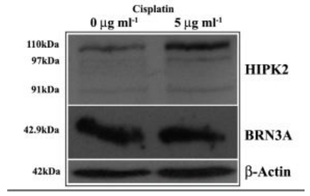




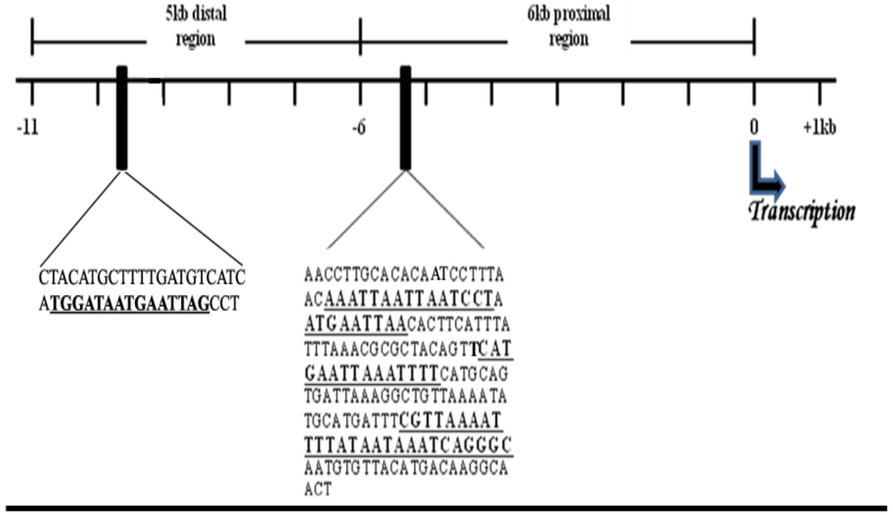


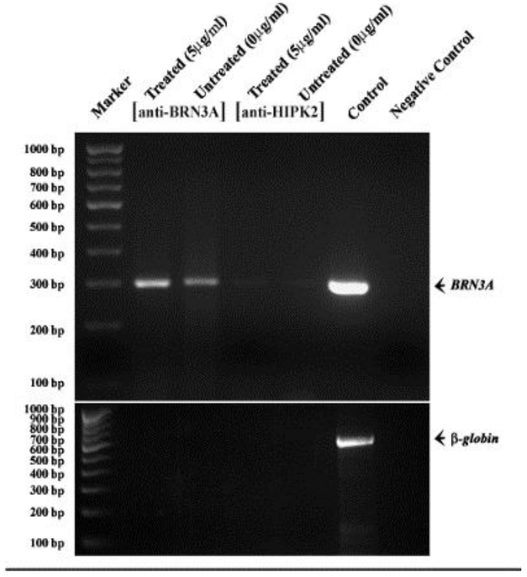






"BRN3A is not supressed" even on elevating and activating the endogenous HIPK2 through chemotherapeutic treatment in cervical cancer cells



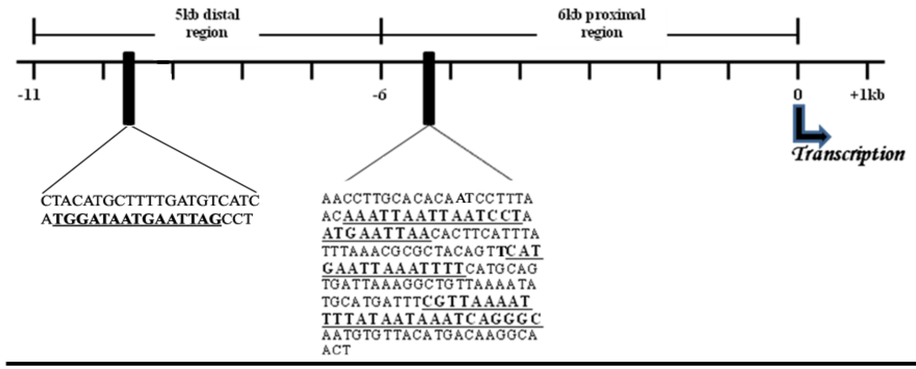


"Autoregulation" appears to be the dominant regulatory mechanism of BRN3A in the uterine cervix

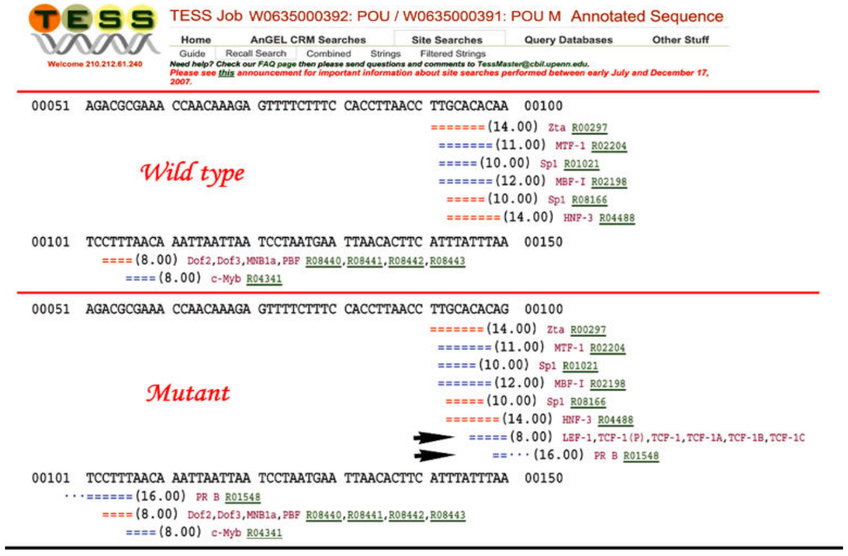
Unaltered BRN3A might be responsible for chemoresistance in cervical cancer cells

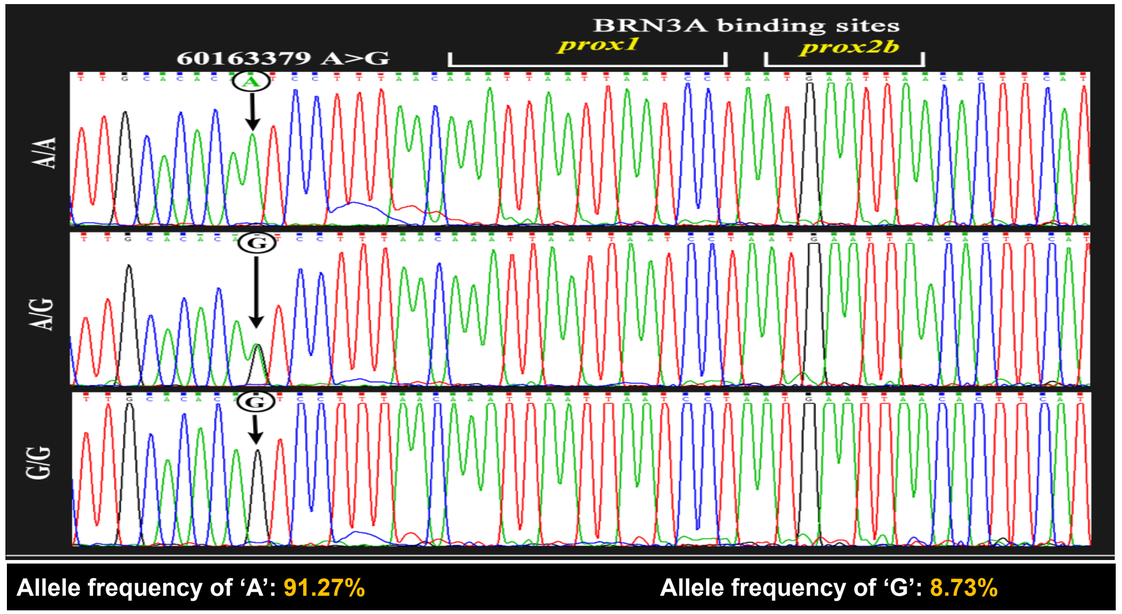
Das Purkayastha and Roy, 2011

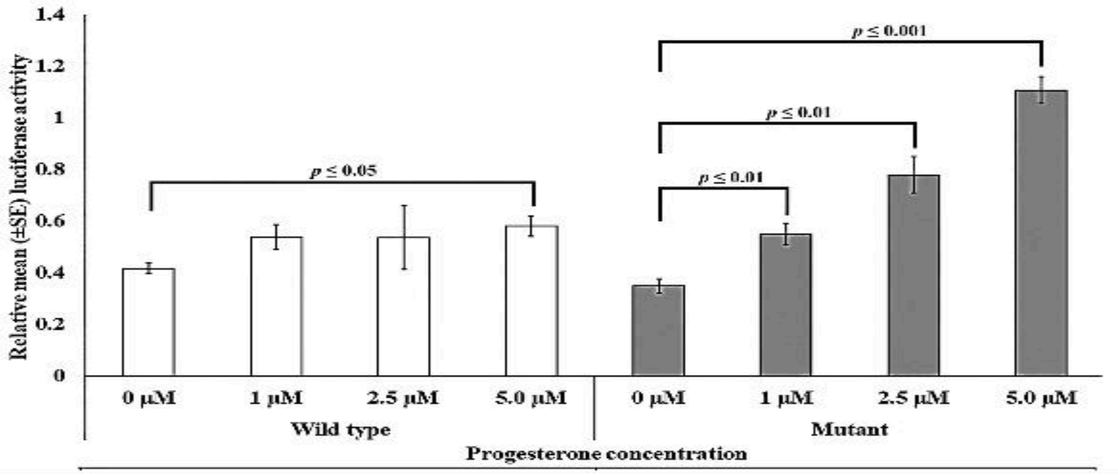
Does any other transcription factor influence *BRN3A*?



Genotype of BRN3A (Enhancer)	Control (in %)	Case (in %)	P (OR (95% CI))
A/A	88.9	61.1	-
A/G	9.3	33.3	0.0098*
G/G	1.8	5.6	0.2953







Increase in luciferase activity in g.60163379 A>G variant in progesterone dependent manner

Manuscript under revision

How does BRN3A exert the tumourogenic activity?

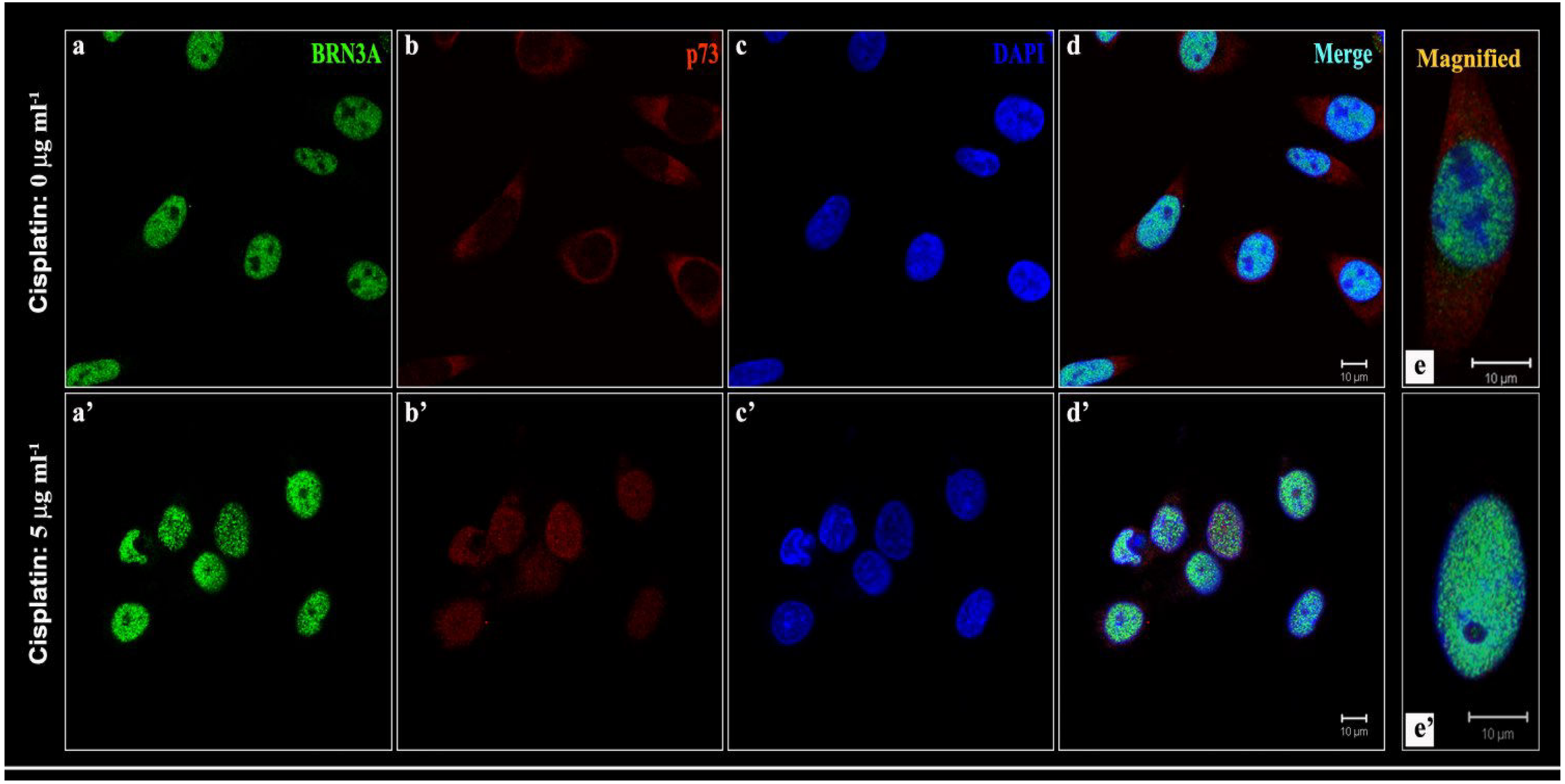
- p73 is a member of the p53-family of proteins
- E6 protein of HPV physically interacts with p53 and subsequently directs it towards proteolysis, but the same E6 is unable to interact with p73

- c-Jun NH_2-terminal kinase (JNK) is a member of the mitogen-activated protein kinase (MAPK) family
- It is also known as stress-activated protein kinase (SAPK) as the kinase gets activated when the cell experiences genotoxic stress

- Brn3a has been observed to modulate the function of p73 in ND7 neuron derived cell line

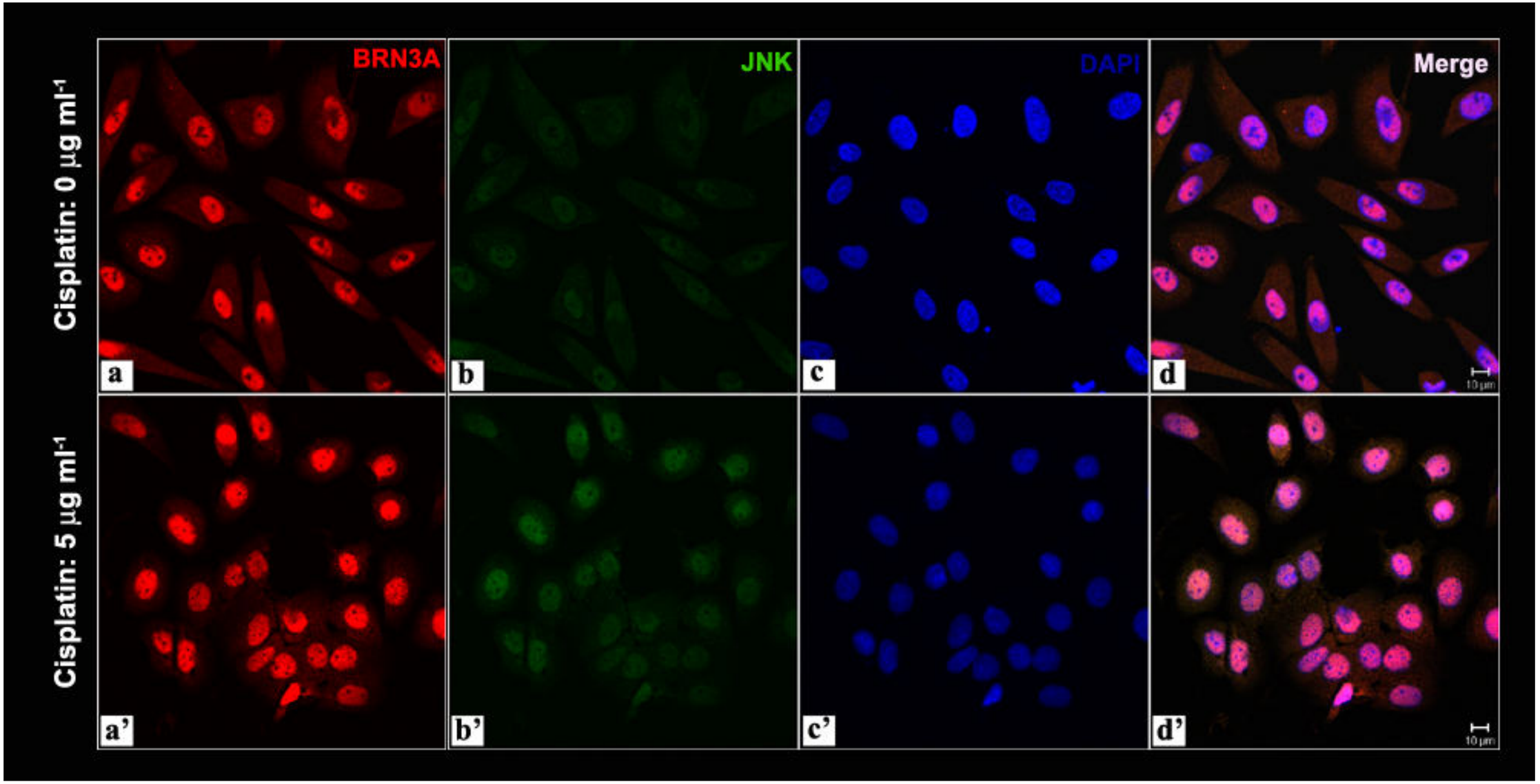
- JNK under the influence of oncoproteins also signals the transformation of cells

Does p73 and JNK get associated with the oncoprotein,BRN3A, in uterine cervix cancer?



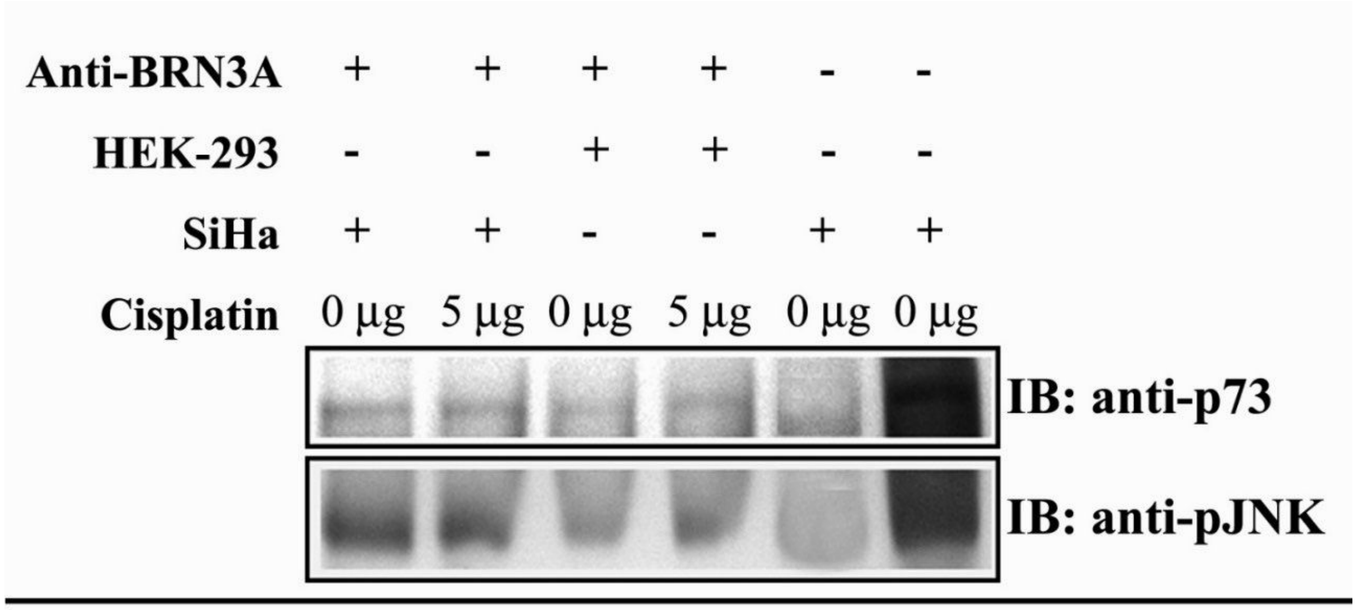
Co-localization of BRN3A and p53-family member, p73, in SiHa cells

Das Purkayastha and Roy, 2011



Das Purkayastha and Roy, 2011

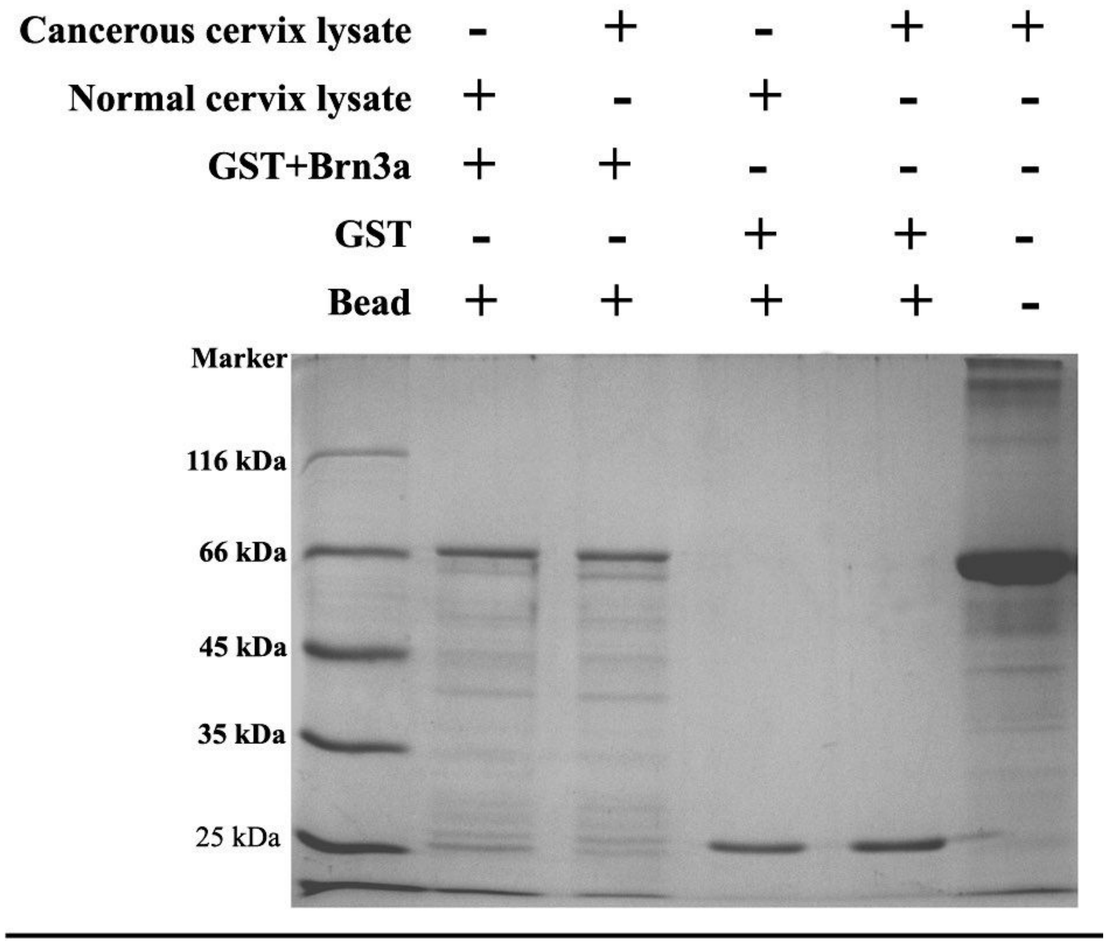
Co-immunoprecipitation with anti-BRN3A antibody for the precipitation of p73 and activated-JNK in uterine cervix cancer cells for knowing their association



The oncogenecity of BRN3A in uterine cervix cancer cells is through its association with p73 and activated-JNK

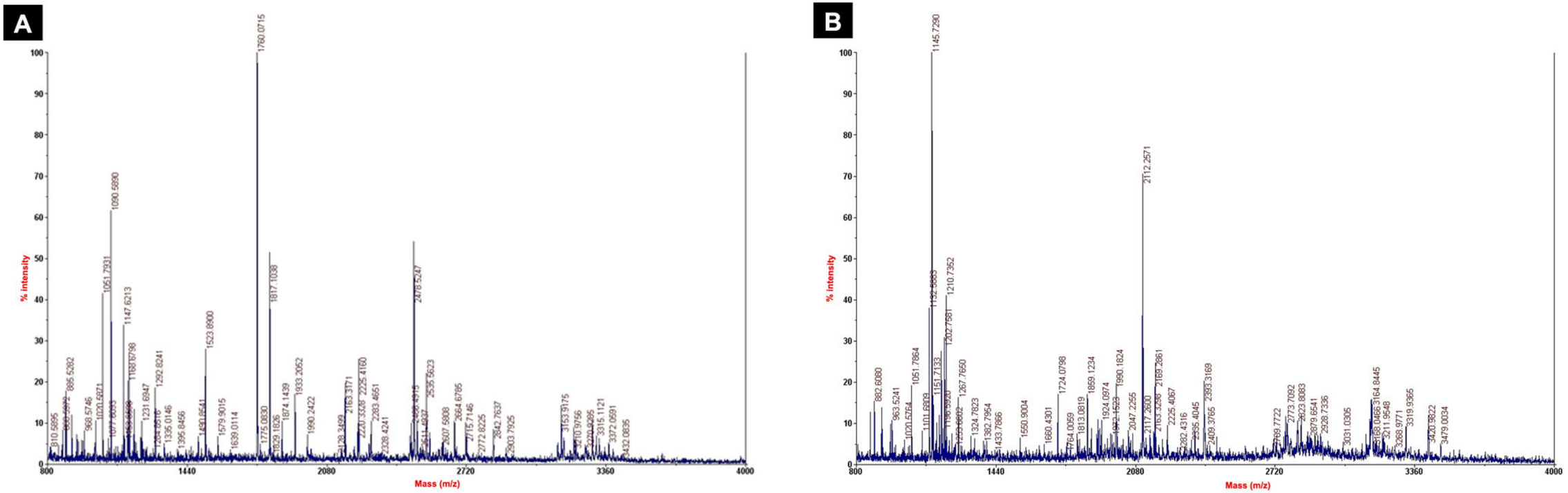
Are there any more interacting partners of BRN3A present in the uterine cervix?

Identification of BRN3A interacting partners in the uterine cervix



Different interactors of BRN3A were isolated from the nuclear extract of normal and cancerous uterine cervix through GST pull-down assay. The interacting partners of BRN3A were then separated in an SDS-PAGE. The peptides were extracted through in-gel trypsin digestion and processed for MALDI-TOF

Unpublished data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2225.4067
2393.3169
2623.8083
2773.7092
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3031.0305
3211.9548
3319.9365
3479.0034

- Plausible interacting partners of homeodomain transcription factor, BRN3A, in the uterine cervix cells:

1. Synaptonemal complex protein 2
2. POU domain, class 4, transcription factor 2 **(BRN3B)**
3. **Cervical cancer suppressor gene-4 protein (MAPKK kinase)**
4. Spermatogenesis-associated protein 24
5. **Mitogen-activated protein kinase 10 isoform 4 (JNK3)**
6. **AML 1**
7. Mutant Pax6
8. Friend leukemia integration 1 transcription factor isoform 2 **(Proto-oncogene Fli-1)**
9. Histone H1.3 Friend leukemia integration 1 transcription factor isoform 2

Summary of the results

- Preliminary investigation of the oncoprotein, BRN3A, reveals that it is mostly active in the uterine cervix for the trans-activation of its target genes

- In the uterine cervix, there is no physical association of homeodomain transcription factor, BRN3A, and co-repressor of homeodomain proteins, HIPK2

  The lack of interaction between homeodomain transcription factor, BRN3A, and co-repressor of homeodomain proteins, HIPK2, is one of the conceivable reason for the unaltered status of BRN3A expression under the condition of elevated HIPK2 in uterine cervix cancer cells

- BRN3A binds to its own enhancer *in vivo* for the autoregulation, with the consequence of upholding its steady state in genotoxically stressed uterine cervix cancer cells

  The insensitivity of the oncoprotein, BRN3A, to the chemotherapeutic treatment suggests that it is one of the plausible mechanisms of chemoresistance

- Interestingly, the 60163379 A>G may be argued to be decreasing the risk of high-risk HPV-induced cancer of the uterine cervix as well

- Amongst the intricate associations of cellular factors in the tumorigenicity of uterine cervix cells, two vital cellular regulators, p73 and JNK has been observed to be associated with BRN3A
  These associations may be contemplated in assisting BRN3A for the immortalization and transformation processes in uterine cervix cells

Conclusion

The propensity of development of uterine cervix tumour and subsequently into malignant lesions is guided by the homeodomain transcription factor, BRN3A

This host cellular factor not only facilitates high-risk HPV infection in the uterine cervix, but this study elaborates the projected risk and role of BRN3A in development of uterine cervix cancer in general

BRN3A may be entitled as the key component in uterine cervix cancer & specifically in HPV-induced CaCx

“Prevention is better than cure” — possible in cancer !!!

Scientific Credential

Scientific Score

- **h-index —** 8
- **i-10 index —** 8

Manuscript (Under-submission &/ or -preparation)

- **BP Das Purkayastha***, A Prakash*, *et.al.*, (**Under review**). Identification of a novel variation in the sensory enhancer of oncogene, BRN3A, in the uterine cervix (***contributed equally**)
- **BP Das Purkayastha***, A Chelluboyina*, *et.al.*, (**Under preparation**). Identification of novel disease susceptibility genomic factors in familial syndrome of esophageal adenocarcinoma and Barrett esophagus (***contributed equally**)
- A Baru, S Sharma, **BP Das Purkayastha**, *et.al.*, (**Under review**). AXTEX-4D™: A novel 3D *ex vivo* platform for preclinical investigations of immunotherapy agents
- A Baru, S Mazumdar, P Kundu, S Sharma, **BP Das Purkayastha**, *et.al.*, (**Under review**). Recapitulating tumor microenvironment using preclinical 3D tissueoids model for accelerating cancer research and drug screening
- S Mazumdar, R rastogi, A Sundale, K Arora, N M Arora, **BP Das Purkayastha**, *et.al.*, (**Under review**). PRAK-03202: A triple antigen VLP vacine candidate against SARS CoV-2

Peer-reviewed Prime Research

- **BP Das Purkayastha**, ER Chan, D Ravillah, L Ravi, R Gupta, MI Canto, JS Wang, NJ Shaheen, JE Willis, A Chak, V Varadan, K Guda (**2020**). Genome-Scale Analysis Identifies Novel Transcript-Variants in Esophageal Adenocarcinoma, *Cellular and Molecular Gastroentrology and Hepatology* (**IF-9.2**), 10 (3), 652-654, e17. (https://pubmed.ncbi.nlm.nih.gov/32344180)
- G Weber*, **B Purkayastha***, L Ren, S Pushpakumar and U Sen (**2018**). Hypertension exaggerates renovascular resistance via miR-122-associated stress response in aging, *Journal of Hypertension* (**IF-4.1**), 36(11), 2226-2236. (***contributed equally**). (https://pubmed.ncbi.nlm.nih.gov/30256768/)
- RK Sharma, ZS Cheda, **BP Das Purkayastha**, **et.al**., (**2016**). A spontaneous metastasis model reveals the significance of claudin-9 overexpression in lung cancer metastasis, *Clinical & Experimental Metastasis*, (**IF-3.1**), 33(3), 263-275. (https://pubmed.ncbi.nlm.nih.gov/26669782)
- **BP Das Purkayastha** and Jagat Kumar Roy, (**2014**). Cancer cell metabolism and developmental homeodomain/ POU domain transcription factors: A connecting link, *Cancer Letters* (**IF-7.4**), 356; 2A, 315-319. (https://doi.org/10.1016/j.canlet.2014.05.015)
- **BP Das Purkayastha** and Jagat Kumar Roy, (**2011**). Molecular analysis of oncogenicity of the transcription factor, BRN3A, in cervical cancer cells, *Journal of Cancer Research & Clinical Oncology* (**IF-3.6**), 137, 1859-1867. (https://pubmed.ncbi.nlm.nih.gov/21928122)

Significant Discovery

- **Novel mRNA, Collagen type X alpha 1 chain var1 (COL10A1var1) mRNA** --- Submitted to the NCBI GenBank **(Accession Number: MN308081) (https://www.ncbi.nlm.nih.gov/nuccore/MN308081?report=GenBank**

- **A single nucleotide variant in the regulatory region of BRN3A** --- Submitted to NCBI SNP database (dbSNP). (Accession Number.: ss289117722)

Manuscript from collaborative projects

- NK Singh, **BP Das Purkayastha**, *et.al.*, (2012). *Journal of Applied Polymer Science* (**IF-2.5**), 127, 2465-2474. (https://doi.org/10.1002/app.37954)
- NK Singh, **BP Das Purkayastha**, *et.al.*, (2012). *Journal of Material Chemistry* (**IF-11.3**), 22, 17853-17863.https://doi.org/10.1039/C2JM32340K
- A Mishra, **BP Das Purkayastha**, *et.al.*, (2012). *Journal of Physical Chemistry C* (**IF-4.2**), 116, 2260–2270. (https://doi.org/10.1021/jp210560s
- NK Singh, **BP Das Purkayastha**, *et.al.*, (2011). *Journal of Materials Chemistry* (**IF-11.3**), 21, 15919-15927. (https://pubs.rsc.org/en/content/articlelanding/2011/jm/c1jm12427g)

GRANT/ FELLOWSHIP/ AWARD

- **DBT−Research Associateship in Biotechnology and Life Sciences − Indian Institute of Sciences (Bangalore, India) and Department of Biotechnology, Government of India, (New Delhi, India)** (Period: **2013-2014**) for the project entitled: Diagnosis of HPV infection in asymptomatic women of NE−India and development of a novel therapeutic approach for the control of HPV infection and subsequent tumorigenicity
- **Senior Scholarship by Lady Tata Memorial Trust, Mumbai, India** (Period: **2010−2012**) for the project entitled: Study of the regulation of BRN3A in cancer of the uterine cervix

***MEMBERSHIP*:**

- Life Member, Indian Society of Cell Biology, INDIA
- Student Member, American Heart Association, USA
- Student Member, Indian Society of Human Genetics, INDIA

***VOLUNTARY SERVICES & EXTRA-CURRICULAR ACTIVITY*:**

- Voluntarily judged scientific posters on October 11, 2016 in the scientific event, 'Research over Louisville' at University of Louisville (Kentucky), USA
- Voluntarily judged scientific posters on October 27, 2015 in the scientific event, 'Research over Louisville' at University of Louisville (Kentucky), USA
- Student Member of the NGO, 'Environmental Society of North Assam', Assam, INDIA

Thank you

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Research Activity

Academic research experience

January 2013 - December 2014

Study on the transcription factor, BRN3A, in etiology of uterine cervix cancer

Silchar (Assam), India

Academic research experience

March 2015 - February 2017

Molecular mechanism of metastasis & chemoresistance in aggressive lung cancer

Louisville (Kentucky), USA

Academic research experience

February 2017 - January 2020

Understand the molecular genetics involved in the progression of Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC)

Cleveland (Ohio), USA

Industrial research experience

January 2020 - May 2021

Development of 3-D mammalian cell-culture model using a patented technology

Gurgaon (HR), INDIA