ROLE OF HISTONE DEACETYLASES (HDACs) IN HUMAN CANCER

RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIEMENT OF

MASTER OF SCIENCE IN LIFE SCIENCE

By

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CERTIFICATE

This is to certify that the thesis entitled **"Role of Histone deacetylases (HDACs) in human cancer"** which is being submitted by **Ms. Minashree Jena,** Roll No. **409LS2043,** for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafied research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

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DECLARATION

I, Minashree Jena, hereby declare that this project report entitled **"Role of Histone deacetylases (HDACs) in human cancer"** is the original work carried out by me under the supervision of Dr. Samir K. Patra, Department of Life Science, National Institute of Technology Rourkela (NITR), Rourkela. To the best of my knowledge and belief the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree or diploma.

Minashree Jena

Place:

Date:

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Finally all credits goes to my parents and my friends for their continued support.

And to all mighty, who made all things possible……..

MINASHREE JENA

CONTENTS

ABSTRACT

Histone modification is the form of epigenetic control, which is quite complex and diverse. The regulation of gene expression and the balance of histone acetylation and deacetylation is an epigenetic layer can be maintained by the post translational modifications of core histones. Histone acetylation is an important mechanism for the control of eukaryotic transcription. Acccumulated evidence shows that aberrant regulation of histone deacetylase is one of the major causes of development of human malignancies.HDAC modulates gene expression by chromatin remodelling during malignant transformation and represses transcription by deacetylating histone. Altered expression and mutations of genes that encodes HDACs have been linked to tumor development since they both induce aberrant transcription of key genes regulating important cellular functions such as cell proliferation, cell-cycle regulation and apoptosis. Among different isoenzymes of HDAC, how HDAC1 is causing carcinogenesis in solid tumors is little unknown. The aim of this study was to check the expression of HDAC1 for the regulation of malignant behavior in lymph node cancer cell.HDAC1 showed expression in the cancer cell indicating the involvement of HDAC1 in the inactivation of certain genes playing role in lymph node metastasis.

Key words: histone modification, HDAC, chromatin remodeling, isoenzymes , malignancies.

1. INTRODUCTION

Histone deacetylases are the class of enzymes which are involved in various epigenetic mechanisms. The history of epigenetics is linked with the study of evolution and development. But during the past 50 years, the meaning of the term "epigenetics" has itself undergone an evolution that parallels our dramatically increased under-standing of the molecular mechanisms underlying regulation of gene expression in eukaryotes. The word epigenetics can be defined as "the study of changes in phenotype that can not be explained by changes in DNA sequence" (Riggs *et al.*, 1996). Until the 1950s, however the word epigenetics was used in an entirely different way to categorize all of the developmental events leading from the fertilized zygote to the mature organism-that is, all of the regulated processes that, beginning with the genetic material, shape to the final product (Waddington, 1953). In other words epigenetics change influence the phenotype without changing the genotype as shown in fig: 1.

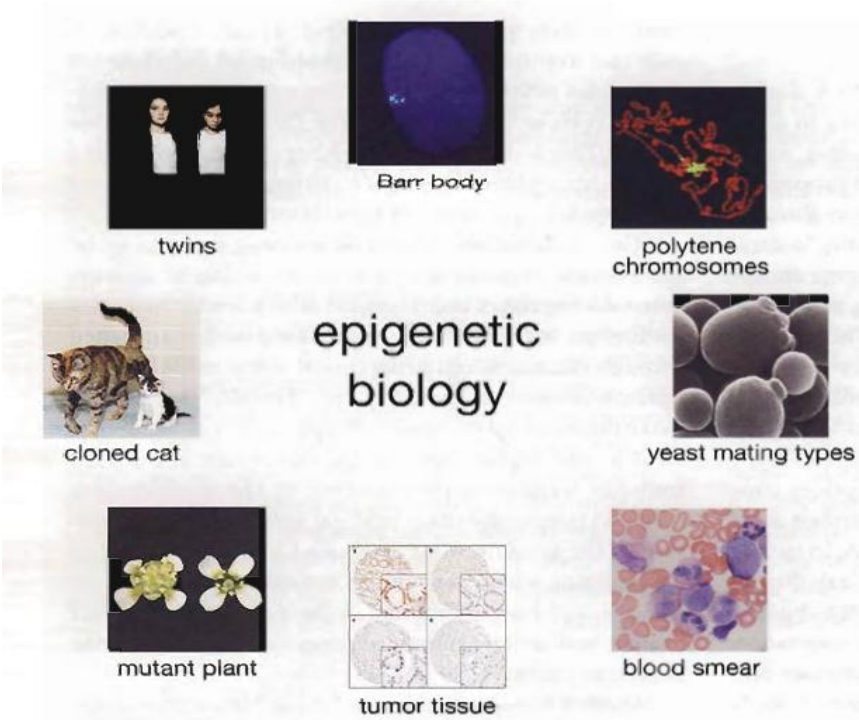


Fig.1: General description about epigenetics (adapted from *Epigenetics* by C.David Allis)

C.H Waddington in 1942 coined the term **Epigenetics** as a portmanteau of the words genetics and epigenesist .It is an old word ,more recently been used to describe the differentiation of cells in embryonic development from their initial totipotent state. The usage of the word in scientific discourse is more narrow , that refers to heritable traits (over rounds of cell division and sometimes transgenerationally) that do not involve changes in DNA sequence .The Greek word "*epi"* represents that epigenetic traits exist on top of or in addition to the traditional molecular basis for inheritance.

This word is having similarity with the word "genetics" that has generated many parallel usages. The word "epigenome" is a parallel to the word "genome" and it refers to the epigenetic state of a cell in whole. The phrase "genetic code" has also been adapted the "epigenetic code" has been used to describe the set of epigenetic features that create different phenotypes in different cells. The "epigenetic code" could represent the total state of the cell, with the position of each molecule accounted through a a diagrammatic representation of the gene expression *i.e.*, **epigenomic map**.

1.1 EPIGENETIC PROCESSES

The various epigenetics processes are mainly

- DNA methylation
- Histone modification
- RNA interference

The psychologist **Erik Erikson** used the term *epigenetics i*n his theory of psychosocial development. That usage, however, is of primarily historical interest.

1.2 MOLECULAR BASIS OF EPIGETICS

The molecular basis of epigenetics is complex that involves modifications of the activation of certain genes, but do not involve in changing the basic structure of DNA. In addition to this, the chromatin proteins associated with DNA may be activated or silenced. This is the reason why the differentiated cells in a multi-cellular organism express only the genes which are necessary for their own activity. When cells divide, they

preserve epigenetic changes. Within the course of one individual organism's lifetime most epigenetic changes tries to express themselves, but, if a mutation in the DNA has been caused in sperm or egg cell that results in fertilization, then some epigenetic changes are inherited from one generation to the next. The question arises here is of whether or not the alteration of the basic structure of its DNA is due to epigenetic changes in an organism - Lamarckism.

1.3 EPIGENETIC EFFECT IN HUMANS

- **Genomic imprinting and related disorders**

  The phenomenon in mammals in which both father and mother contribute different epigenetic patterns for specific genomic loci in their germ cells,is named as genomic imprinting. The various disorders associated with genomic imprinting are: Angelman syndrome, Prader willi syndrome, Beckwith-wiedemann syndrome.

- Transgenerational epigenetic observations
- Cancer and developmental abnormalities

1.5 HISTONE DEACETYLASES (HDACs)

Classes of enzymes that remove acetyl groups from an ε-N-acetyl lysine amino acid on a histone are **Histone deacetylases (HDAC)**. Histone acetyltransferase acts opposite to that of HDAC proteins that are now also being referred to as lysine deacetylases (KDAC). The balance between the opposing activities of histone acetyl transferases and histone deacetylases (HDACs) results steady-state levels of acetylation of the core histones. Increased transcriptional activity is due to increased levels of histone acetylation (hyperacetylation), whereas decreased levels of acetylation(hypoacetylation) are associated with repression of gene expression.

1.6 GENERAL OVERVIEW

Classification

Based on function and DNA sequence similarity can be categorized into four groups . Trychostatin A (TSA) is a HDAC inhibitor but it does not shows any effect on classII HDACs which belongs to a, family of NAD+-dependent proteins. Reduced potassium dependency 3 (Rpd3), that corresponds to Class I- histone deacetylase1 (hda1), Class II- and silent information regulator 2 (Sir2), Class III are three groups which are homologues to these proteins and are found in Yeast. The fourth group is considered an a typical category of its own, based solely on DNA sequence similarity to the others. HDAC 1, 2, and 8 are found primarily in the nucleus that will be included under Class I HDACs, whereas HDAC 3 is membrane-associated i.e, found in both the nucleus and the cytoplasm .ClassII HDACs (HDAC 4, 5, 6, 7, 9 and 10) share domains with similarity to HDAC1,another deacetylase found in yeast. A new member of the HDAC family has been identified recently,. All the necessary features for this HDAC11 to be designated as a HDAC are found to be present in it. (Annemieke *et al.*, 2003).

Localization

HDACs should be in the nucleus, where their predominant substrate is found to exert their function. The nuclear localization of HDACs is triggered by the nuclear localization signal (NLS) or via co-localization together with other proteins. The localization of HDAC1 and HDAC2 is exclusively nuclear due to the lack of a nuclear export signal (NES). However, HDAC3 has both a NES and a nuclear import signal, suggesting that HDAC3 can also localize to the cytoplasm. Class II HDACs (HDAC 4, 5, 6, 7, 9, and 10) can shuttle in and out of the nucleus depending on different signals. HDAC 6 is a cytoplasmic, microtubule -associated enzyme which deacetylates tubulin, Hsp90, and cortactin, and forms complexes with other partner proteins, and is, therefore, involved in a variety of biological processes.

Revolutionary relationship between the HDAC

- Evolutionarily, the classes I HDACs are related to yeast RPD3 and the class II HDACs are related to the yeast HDAC enzyme.
- RPD3 is most related to HDAC1 and HDAC2; HDAC1is most closely related to HDAC6.
- From the phylogenetic tree, it can be concluded that HDAC9a, HDAC9b and HDAC9c/HDRP form a distinct group with in classII that seem to be less related to the members of class II.
- HDAC11has been placed in other class because it has not shown enough identity with classI or classII HDACs.

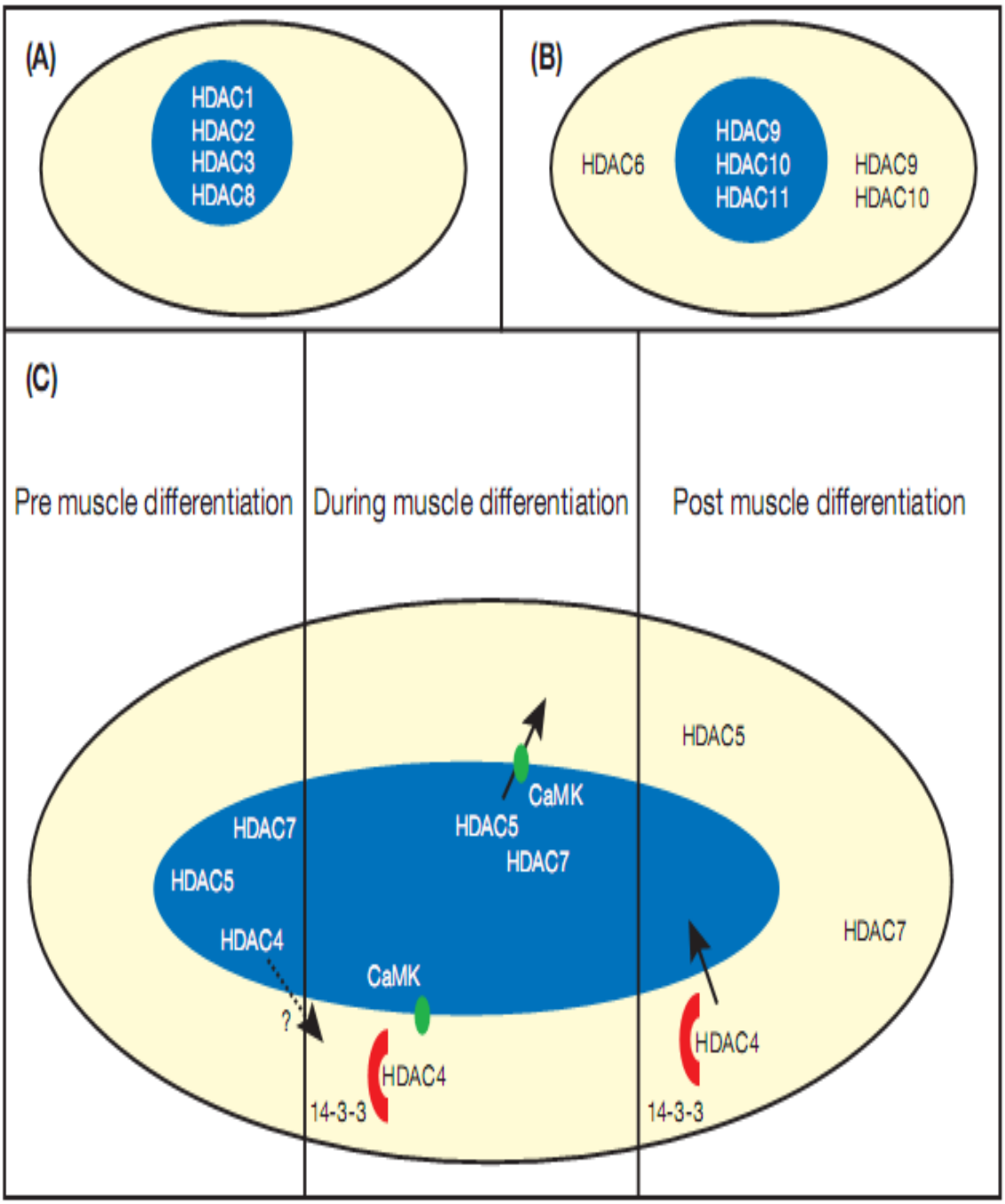


Fig. 2: Localization of different HDACs in which the nucleus is depicted in blue.14-3-3 protein (red) can retain HDAC4/5 orHDAC7 in the cytoplasm when they are phosphorylated (Annemieke *et al.*, 2003). CaMK (green) is involved in nuclear export via the calcium/calmodul in signalling pathway. Subcellular localization of (A) classIHDACs and (B) classII HDACs are shown,(C) Shuttling of HDAC4,HDACs5 and HDACs7 during muscle differentiation .

1.7 MECHANISM OF ACTION

HDAC enzymes remove the acetyl group from the histones. The space between the nucleosome and DNA is decreased due to hypo acetylation and the DNA is compactly wrapped around it. The accessibility of transcription factors is decreased by tighter wrapping of the DNA which leads to transcriptional repression, as given in figure 1.The catalytic domain of HDAC is formed by a stretch of approximately 390 amino acids consisting of a set of conserved aminoacids.The active site consists of a gently curved tubular pocket with a wider bottom. Removal of an acetyl group occurs via a charge relay system consisting of two adjacent histidine residues, two aspartic residues (located approximately 30amino acids from the histidines and separated by approximately 6 aminoacids) and one tyrosine residue (located approximately 123aminoacids downstream from the aspartic residues). An essential component of this charge-relay system is the presence of a Zn^{++} ion as shown in fig. 3 and 4. This atom is bound to the zinc binding site on the bottom of the pocket. Other co-factors are required for HDAC activity. HDAC functions by displacing the zinc ion and thereby making the charge-relay system dysfunctional. TSA is the most potent reversible HDACi currently known, with an IC_{50} in low nano molar range. TSA, with its hydroxamic acid group and its five-carbon atom linker to the phenyl group, has the optimal conformation to fit into the active site.

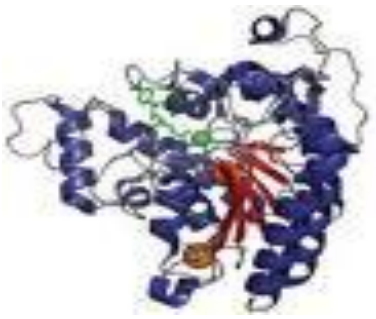


Fig. 3: Zinc dependent HDAC

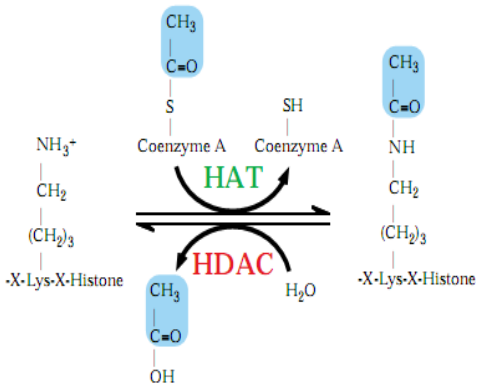


Fig.4:HAT AND HDAC ACTIVITY

1.8 HDAC INHIBITOR (HDACi)

Recent studies shows that HDACi inhibits HDAC by blocking the access of HDAC to the active site (reversible or irreversible).There is many inhibitors but most potent discovered so far are Trychostatin (TSA) which is a fermentation product of Streptomyces. Originally TSA was used as an anti-fungal agent, but later it was discovered to have potent proliferation inhibitory properties with cancer cells.

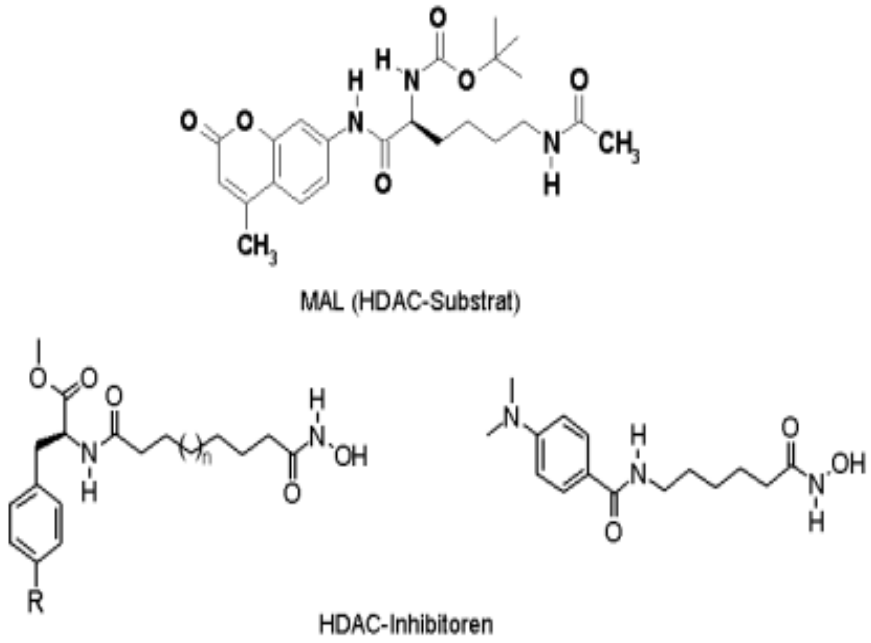


Fig. 5: Activity of HDAC inhibitors

2. REVIEW OF LITERATURE

2.1 Role of HDAC and N-CoR in sin3 mediated transcriptional repression

sin3 can function as a transcriptional repressor without involving any chromatin regulators. The activity of component are convincing of the basal transcriptional machinery are affected by the sin3 associated proteins. The function of sin3 as a repressor is known because findings show that transcriptional activation of some genes is reduced in case of sin3 null mutants. The interaction between msin3 and N-CoR mediate the transcriptional silencing by nuclear hormone receptor. Myc activities require interaction with mammalian sin3a and sin3b protein for mediating Mxi1- inhibition, which have been proposed to act as scaffolds for additional co-repressor factors. The identification of two such sin-3 associated factors, the nuclear receptor co-repressor (NCo-R) and histone deacetylase (HDAC1), provides a basis for Mxi1/sin3 induced transcriptional repression and tumor suppression.

Transcriptional repression by msin3A and msin3B

Studies shown that when mammalian protein msin3Blf and msin3Bsf were fused to GAL4 DNA binding domain (GAL4), it can repress the basal transcription of a chloramphenicol acetyl transferase which bears five GAL4 DNA binding sites upstream either major late promoter (MLP) or thymidine kinase promoter (TK).When the regions of msin3A containing only PAH1 and PAH2 (paired amphipathic helical) were fused to GAL4 domain similar results were obtained.

HDAC1 interact with msin3Blf but not with msinBsf:

It has been suggested that the C- terminal region of PAH2 is integral to tumor suppression mediated by msin3B. From the genetic analysis of yeast it has been found that the link between histone acetylation factors and tumor prone conditions shows a tight functional relationship between sin3 and RPD3 (a yeast homologue of the human histone deacetylase HDAC1). When low-stringency co-immunoprecipitation was done between HDAC1 and msin3B to know whether there is any physical interaction between these two in mammalian cell, it has been found that the long form of msin3B(PAH3+4) possesses

potent anti-oncogenic activity and is sufficient for the interaction between HDAC1 and msin3B when linked directly to Mxi1 (Alland *et al.*, 1997).

2.2 Role of HDACs in cancer (Santiago Ropero,Manel Esteller,2007)

- Deregulation of DNA methylation and posttranslational histone modification, especially histone acetylation is a typical characteristic of human cancer with fatal consequences such as that of gene transcription-deregulation.
- Data studies from a panel of normal tissues, primary tumors and human cancer cell lines indicate that a loss of acetylated Lys16 (K16-H4) and tri-methylated Lys20 (K20-H4) of histone H4 is a common event in human cancer associated with hypomethylation of repetitive sequences.
- A study of gastro intestinal tumors concluded that the decrease in histone acetylation is not only involved in initiation of tumor genesis but also in tumor invasion and metastasis.
- Although there are no conclusive data about the pattern of HDAC expression in human cancer, there are a number of studies showing altered expression of individual HDACs in tumor samples.
- An increase in HDAC1expression in gastric, prostate, colon, and breast carcinomas.
- Over expression of HDAC2 has been found in cervical, and gastric cancers, and in colorectal carcinoma with loss of APC expression.
- Other studies have reported high levels of HDAC3 and HDAC6 expression in colon and breast cancer specimens, respectively.
- Different findings suggest that the transcriptional repression of tumor suppressor genes by over expression and aberrant recruitment of HDACs to their promoter region could be a common phenomenon in tumor onset and progression.
- The cyclin-dependent kinase inhibitor p21WAF inhibits cell-cycle progression and whose expression is lost in many different tumors.
- In some tumors, p21WAF1 is epigenetically inactivated by hypoacetylation of the promoter, and treatment with HDAC inhibitors leads to the inhibition of tumor-cell growth and an increase in both the acetylation of the promoter and gene expression.
- It has been found that when the transcription factor Snail recruits HDAC1, HDAC2,and the co repressor complex mSin3A to the E-cadherin promoter, its expression is repressed.

- Down regulation or loss of function of E-cadherin has been implicated in the acquisition of invasive potential by carcinomas , and so the aberrant recruitment of HDACs to this promoter may have a crucial role in tumor invasion and metastasis.
- Class I HDACs are involved in transcription-repression mediated by retinoblastoma (Rb) protein. The lost of HDAC2 function could induce hyperacetylation and re-expression of genes regulated by Rb and crucial functions in cell cycle regulation.

2.3 HDAC1 GENE

- HDAC1 belongs to class I HDACs having the catalytic domain on the N-terminus which forms the major part of the protein.
- HDAC1 and HDAC2 remains inactive when they are produced by recombinant techniques, these cofactors are necessary for the activity of HDAC.
- In vivo, HDAC1 and HDAC2 only display activity with in a complex of proteins.
- Proteins necessary for modulating their deacetylase activity and for binding DNA are present in these complexes. Together with proteins they mediate the recruitment of HDACs to the promoters of genes.
- Three protein complexes Sin3v, NuRD (nucleosome Remodelling and deacetylating) and Co-REST that have been characterized with both HDAC1 and HDAC2.
- Both the Sin3 complex (named after its characteristic element mSin3A) and NuRD complex consist of a core complex that constitutes HDAC1, HDAC2, Rb-associated protein 48 (RbAp48, which binds histone H4 directly) and RbAp46.
- The core complex cannot give maximum HDAC activity and needs cofactors additionally.
- HDAC1 and HDAC2 can also bind directly to DNA binding proteins such as YY1 (Yin and Yang1, a cellular nuclear matrix regulatory protein), Rb binding protein-1 and Sp1 apart from functioning through these complexes.
- In addition to the regulation of HDAC1 and HDAC2 activity by the availability of co-repressors, a second means of regulating activity is via post-translational modifications.
- Both activity and complex-formation are regulated by phosphorylation. HDAC1and HDAC2 are phosphorylated at a low steady-state level in resting cells.

- Hyper Phosphorylation of HDAC1 and HDAC2 leads to a slight but significant increase in deacetylase activity and at the same time to disruption of complex-formation between HDAC1 and HDAC2.
- When hypo phosphorylation of HDAC1 and HDAC2 occurs, the activity of HDAC1 and HDAC2 decreases, but complex-formation is increased.
- Maintenance of HDAC activity at a certain optimal level is done by apparent contradictory consequences of Phosphorylation. Mutational analysis of HDAC1 shows that Ser^{421} and Ser^{423} are crucial Phosphorylation sites; when they are mutated,complex-formation is hampered and HDAC activity is decreased (Fig:11)
- Although HDAC1 has been mostly studied in the context of cancer, recent evidence strongly suggests that it plays critical roles in cellular senescence, aging of the liver, myelination, and adult neuro genesis. (Annemieke, *et al.,* 2003)

2.4 **Over expression of HDAC1 proliferates breast cancer by negative regulation of estrogen receptor α.**

- The interaction between17 β-estradiol and estrogen receptor alpha (ER-α) plays an important role in breast carcinogenesis and breast cancer treatment.
- ER- α is a critical growth regulatory gene in breast cancer and its expression level is tightly linked to the prognosis and treatment outcomes of breast cancer patients.
- For breast cancer progression, loss of ER-α expression in breast epithelial cells is critical.
- The molecular mechanisms for this loss, are still poorly defined.
- Alteration of chromatin assembly and tumorigenesis are due to histone deacetylases (HDACs) implication.
- It has been showed that histone deacetylase1 (HDAC1) interacts with ER-α in vitro and *in vivo* and suppresses ER-α transcription activity.
- The interaction of HDAC1 with ER-α was mediated by the AF-2 and DBD domains of ER-α.
- An endogenous interaction of HDAC1 with ER-α has been observed in breast cancer cells, which was decreased in the presence of estrogen.
- A possible mechanism for the loss of ER-α in ER-negative breast cancers is cytosine methylation of the ER CpG island in the 5' regulatory region of the gene.

- The ER gene CpG islands extensively methylated in ER–negative breast cancer cells and approximately 50% of unselected primary breast tumors remain unmethylated in normal breast tissue and in many ER-positive tumors and ER-positive breast cancer cell lines.
- Interestingly, over expression of HDAC1 instable transfected MCF-7 clones induced loss of ER-α and significantly increased cell proliferation and colony formation, as compared to the control MCF-7 cells, whereas treatment of stable MCF-7 clones with the HDAC specific inhibitor Trychostatin (TSA) induced re-expression of ER-α mRNAand protein.
- HDAC1 affects breast cancer progression by promoting cellular proliferation in association with a reduction in both ER-α protein expression and transcriptional activity.
- HDAC1 may be a potential target for therapeutic intervention in the treatment of a subset of ER-negative breast cancers (Kawai, Li, and Avraham *et al.*,2003).

3. OBJECTIVE

To clarify the role of HDACs in the aberrant promoter hypoacetylation of TSGs in various human cancers, the expression levels of a number of HDACs in cancer tissues were examined. The expressions of the HDAC1 gene were examined, since these enzymes have a reported role in deacetylation status integrity and have been implicated in the transcriptional regulatory changes of human cancer.

So our main objective was "**Comparative Analysis of histone deacetylase1 Expression in Normal and Human Cancer Tissues"**.

4. MATERIALS AND METHODS

The Human blood was collected from CWS Hospital, Rourkela as normal human tissue and gall bladder and lymph node cancer tissues were collected from Calcutta National Medical College, Kolkata.

(A) Total RNA Isolation:

Reagents and Buffers

- TRIzol Reagents (Sigma),
- Choloroform,
- Isopropanol,
- Ethanol (70%),
- Denaturation Buffer
  - ✓ 50 % deionized formamide,
  - ✓ 2.2 M formaldehyde,
  - ✓ MOPS buffer (pH 7.0),
  - ✓ 6.6 % glycerol,
  - ✓ 0.5 % bromphenol,
- Ethidium Bromide (EtBr),
- Agarose

Protocol:

- 50-100 mg of tissue in a 2 ml tube with 1 ml TRIzol was transferred.
- Homogenized for 60 sec in the polytron.
- 200 µl chloroform was added.
- It was mixed by inverting the tube for 15 sec.
- Incubated for 3 min at room temperature.
- Centrifuged at 12.000 g for 15 min.
- The aqueous phase was transferred into a fresh Eppendorf tube.
- 500 µl isopropanol was added.

- Centrifuged at max. 12.000 g for 10 min in the cold room.
- The pellet was washed with 500 µl 70 % ethanol.
- Centrifuged at max. 7.500 g for 5 min in the cold room.
- The pellet was dried on air for 10 min.
- Then the pellet was dissolved in 50-100 µl DEPC-H_2O.
- Incubated for 10 min at 60° C.
- Spectrophotometric reading was taken.
- Analysed the RNA on a MOPS gel:
    - 1-3 µg RNA was dissolved in 11 µl denaturation buffer.
    - 1 µl Ethidium bromide (1mg/ml) was added and denatured at 65° C for 15 min
    - 1 % agarose gel was loaded in MOPS buffer plus 5 % formaldehyde.
    - The gel was run at 40 V for 4 h.

(B) cDNA Synthesis (rt-PCR):

Regents and Buffer

- 5X First Strand Buffer
- 10mM dNTP Set
- 0.1M DTT
- Random Primers
- RNase OUT Ribonuclease Inhibitor
- Super Script II RNase H- Reverse Transcriptase

Protocol:

- 8µl of total RNA were taken.
- Then 3 µl Random Primers was added.
- 1 µl dNTP mix was added.
- Then vortex and spin downed tube.
- Incubated at 65°C for 5 min.
- Placed tube on ice.
- 4 µl 5X Buffer, 2 µl DTT and 1µl RNAase Out were added.

- Then vortex and spin downed tube.
- Incubated at 42°C for 1 min.
- 1µl SuperScript II RNase H- Reverse Transcriptase was added.
- Incubated at 42°C for 60 min.
- Incubated at 70°C for 15 min.
- 180 µl of molecular grade water was added.
- Nanodrop 1000 was used to measure concentration. Set sample typesetting to Other Sample and the constant to 33.
- Stored at -80°C.

Gene specific PCR

Used Primers:-

Gene	Forward Primer	Reverse Primer
HDAC1	5'-TTCAAGCCGGTCATGTCCAAAG-3	5'-TTTGATCTTCTCCAGGTACTC-3'
β-Actin	5`- TCTACAATGAGCTGCGTGTG-3`	5`- ATCTCCTTCTGCATCCTGTC-3`

(Patra *et al.*, 2001.)

PCR mixture:-(Total 25µl):

- 0.2 µM dNTP- 0.5µl
- 1.5 mM $MgCl_2$- 1.5µl
- 1x PCR Buffer- 2.5µl
- TaqPolymerase (5U/µl)

PCR conditions: - 94°C for 1min,

26 cycles at 94°C for 20s,

57°C for 20s, and

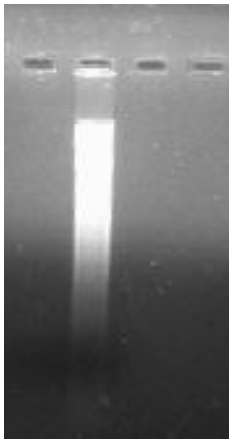
72°C, Followed by an extension step at 72°C for 5min

5. RESULTS AND DISCUSSIONS

RNA EXTRACTION FROM NORMAL TISSUE (BLOOD):

Product	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
Total RNA	598.32	1.45	0.96

Spectrophotometer results of total RNA from blood tissue



(Total RNA in 1% agarose gel)

(Gene specific PCR amplification in blood)

Table 1. Spectrophotometer results of gene specific amplification product from blood

Gene	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
β-actin	1564.12	1.25	1.13
HDAC1	1696.36	0.96	0.83

RNA EXTRACTION FROM CANCEROUS TISSUE:

Table 2. Spectrophotometer results of RNA concentration from Cancer.

Tissue	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
Lymph Node Cancer	917.51	1.61	1.02

Spectrophotometer results of total RNA from cancerous tissue

Lane 1:lymph node cancer

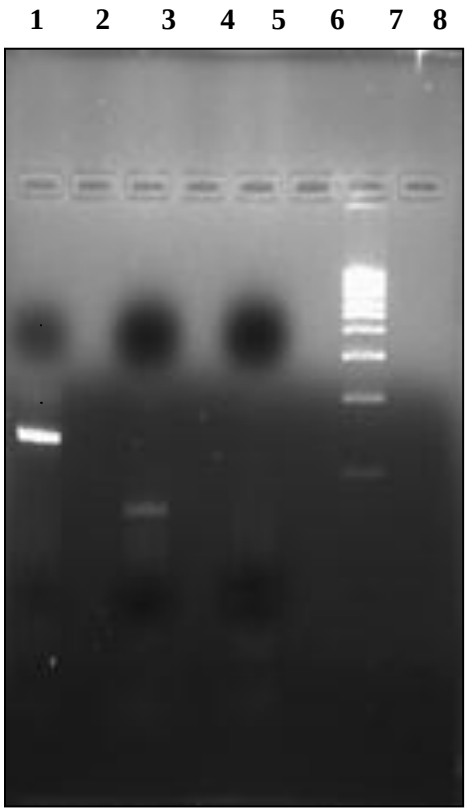
(Total RNA from denaturation gel)

GENE SPECIFIC PCR AMPLIFICATION:

Table 3. Spectrophotometer results of gene specific amplification product from lymph node cancer tissue

Gene	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
β-actin	1998.34	1.331	1.015
HDAC1	1746.0	0.9346	0.1426

Expression of HDAC in lymph node cancer:

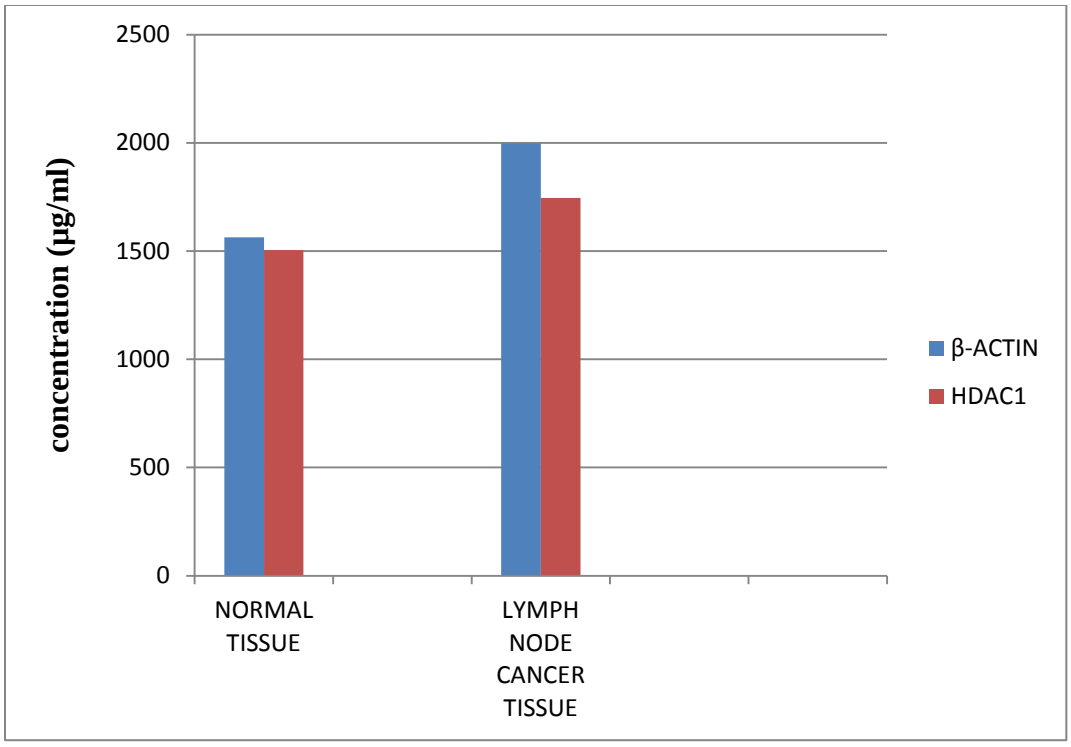


Lane 1: β-actin

Lane 2: HDAC1 gene

Lane 7: DNA ladder (1.5 kb)

Lane 1 shows the expression of β-actin which performs as a house keeping gene and is constitutively expressed. By taking the DNA ladder (lane 7) as a standard the expression of HDAC1(lane 2) has been seen and we can see the expression of HDAC1 suggesting an role in causing lymph node cancer.



Comparative analysis between normal and cancer tissue

After isolation the total RNA from normal blood and Cancerous tissue, we checked their concentration by taking its OD in spectrophotometer, in case of Lymph node cancer the concentration was found to be 917.51µg/ml, which was comparatively less to the other samples reading. Because it was took a lot of time to processing after collecting the sample and also cDNA was not synthesize from the total RNA by rt-PCR method due to some unsuitable condition.

The concentration of the HDAC1 in the normal tissue is 1696.36 µg/ml, and in the lymph node cancer tissue it is 1746.0µg/ml. The activity of the enzyme has been quietly increased in the cancer tissue but as the β-Actin has been maintained as it is only helping in the housekeeping function.

6. DISCUSSIONS

HDACs, appears to be the key enzymes in the regulation of gene expression. HDAC function seems to be regulated by its intrinsic features, abundance, cellular compartmentalization and association with co-factors. Despite extensive investigation of the biological functions of HDACs, a little is known about the expression status of HDACs in lymph node metastasis. Our results clearly showed the increased expression of HDAC1 in lymph node cancer tissue at transcriptional level.So,the increased expression of HDAC1 may causing the down regulation of tumor suppressor gene(TSG),such as p^{53}. These data implies that HDAC1 expression may cause histone hypoacetylation leading to the silencing of several tumor suppressor genes in lymph node cancer.

Now, we know that after methylation of the gene the MeCpG binding proteins will bind the gene which allows the HDACs to bind in their position to de-acetylate the gene that helps in the heterochromatin formation. If we will observe the results we got from the comparison of the normal tissue with the cancer tissue, the expression of the methylating enzymes mainly DNMT1 has been suppressed. From this, we can conclude that the over expression of the HDACs in the cell is leading to the suppression of the DNMTs that finally lay lead to the tumor formation that paves a way to the cancer development.

In conclusion, the present study clearly demonstrated that HDAC1 showed increased expression in lymph node cancer and probably plays a significant role in lymph node cancer.

Further studies:

Further studies using large number of samples and covering other HDACs are needed to identify the cell cycle regulators affected by HDACs and to asses the feasibility in lymph node cancer by using HDACs as prognostic factor. HDAC inhibitor can be used with less cellular toxicity as a cancer therapeutic agent.

7. REFERENCES

1. Annemieke, J.M., Ruijter, D., Albert, H., Gennip, V., Caron, H.N., Kemp, S., Andre B.P. and Kuilenburg, V. (2003). Histonedeacetylases(HDACs): characterization of the classical HDAC family; Biochem. J. 370: 737-749.

2. Alland, L., Muhle, R., Hou, H. (1997). Department of microbiology and immunology.Bronx,New York. Role for N-CoR and histone deacetylase in sin3-mediated transcriptional repression. 225: 238- 247.
3. Liu, H., Hu, Q., Kaufman, A, Ercole A.J. (2001)

4. Wade, P.A. (2001). Transcriptional control at regulatory checkpoints by histone deacetylases :molecular connections between cancer and chromatin.Hum.Mol.Genet. 10, 693-698.

5. Strahl, B.D. and Allis, C.D. (2000). The language of covalent histone modifications. Nature (London) 403, 41-45.

6. Lee, M.G., Wynder, C., Cooch, N. and Shiekhattar, R. (2005). An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. Nature 437: 432-435.

7. Lee, N., Erdjument-Bromage, H., Tempst, P., Jones, R.S. and Zhang, Y. (2009). The H3K4 Demethylase Lid Associates with and Inhibits Histone Deacetylase Rpd3. Mol Cell Biol 29: 1401-1410.

8. Bai,S., Ghoshal,K., Datta,J., Majumder,S., Yoon,S.O., Jacob,S.T. (2005). DNA methyl transferase 3b regulates nerve growth factor induced differentiation of PC12 cells by recruiting histone deacetylase2. Mol.CellBiol.25, 751–766.

9. Prall, O.W., Rogan, E.M., Sutherland, R.L. (1998). Estrogen regulation of cell cycle progression in breast cancer cells. J Steroid Biochem Mol Biol; 65:169–74.

10. Patra, S.K., Patra, A., and Dahiya, R. (2001). Role of histone deacetylase and DNA methyl transferase in human prostrate cancer.

11. Usmani, B.A., Shen,R., Janeczko,M., Papandreou,C.N., Lee W.H., Nelson, W.G., Nelson, J.B. and Nanus,D.M. (2000). Methylation of the neutral endopeptidase gene promoter in human prostate cancer. Clin.CancerRes. 6, 1664–1670.

12. Boyes, J. and Bird, A. (1991). DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell 64:1123–1134.

13. Brehm, A., Miska, E.A., McCance, D.J., Reid, J.L., Bannister, A.J., Kouzarides, T. (1998). Retino blastoma protein recruits histone Deacetylase to repress transcription. Nature 391:597–601.

14. Paranjape, S.M., Kama, R.T., Kadonaga, J.T. (1994). Role of chromatin structure in the regulation of transcription by RNApolymeraseII. Annu Rev Biochem 63:265–297.

15. Hall, J.M., Couse, J.F., Korach, K.S. (2001). The multifaceted mechanisms of estradiol and estrogen receptor signaling. J.BiolChem; 276: 36869–72.