WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 9, 151-180. Review Article ISSN 2277– 7105

ROLE OF JUNK DNA IN ONCOGENETICS

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Article Received on
09 May 2022,

Revised on 30 May 2022,
Accepted on 19 June 2022

DOI: 10.20959/wjpr20229-24559

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ABSTRACT

Junk DNA has long been conceived of by evolutionists as unused DNA from previous evolutionary iterations. According to the selfish or parasitic DNA idea, this DNA survives only due to its ability to duplicate itself, or because it has randomly mutated into a form that is beneficial to the cell. Introns, pseudogenes, mobile or repetitive DNAs are all examples of junk DNA. Many previously discarded DNA sequences have recently gained renewed attention for their roles in genome structure and function, gene control, and fast speciation. On the other hand, there are examples of what appear to be actual junk DNA sequences, or sequences that have lost their activities due to mutational inactivation that could have occurred after the Fall, or by God-ordained temporal restrictions placed on their functions (Walk up, 2000). The preservation of telomere length is required for long-term cell division. Telomerase lengthens telomeres and maintains a telomere length balance that prevents them from becoming dangerously short when they get too short to protect chromosomal ends., and emphysema in people with insufficient telomerase or other telomere gene abnormalities. The ability to annotate enhancers and their activity levels is based on epigenomic characteristics such as the build-up of histone H3 lysine 4 monomethylation and histone H3 lysine 27 acetylation. BD modules bind to acetylated histones in a variety of transcriptional coregulators, influencing chromatin accessibility and transcriptional activation at active enhancers. Epigenetic is a Greek word that means "above and beyond." Conrad Waddington invented the term in 1939 to describe the process by which a population's response to an environmental stimulus is passed down through the generations.

KEYWORDS: junk DNA, BRD4 inhibitors, Histone proteins, Micro RNA, Breast Cancer, Paediatrics Cancer.

INTRODUCTION

What if someone tells you that our DNA has only 10% of information that translate to any particular protein or amino acids whereas more than 90% of our DNA contains sequences which does not translate to anything yet it is there. Just like new mobile that comes with tons of "Bloatware", most of the user are not going to use it yet it is preinstalled on the device which is not only useless it occupies extra space and slows your device. In 1970s we came to know that much like Bloatware our body also comes with preinstalled useless pairs of DNAs popularly known as "JUNK DNA".[1] Before that Biologists had expected that DNA only represent essential information. The lack of correlation between the amount of noncoding DNA and the number of protein-coding genes has created the impression that nongenic DNA is 'junk DNA.' The majority of DNA in eukaryotic genomes is not translated into protein sequence. Noncoding DNA (ncDNA), on the other hand, has received little attention in comparison to proteins. Due to a lack of understanding of its functional relevance, it has been speculated that much nongenic DNA is useless 'junk' or exists solely to duplicate itself. Many eukaryotic genomes contain a substantial proportion of middle-repetitive DNAs and transposable elements (TE), which could be 'selfish' DNA.[2] Maize geneticist Barbara McClintock discovered TEs in the 1940s, and for decades thereafter, most scientists dismissed transposons as useless or "junk" DNA. McClintock, however, was among the first researchers to suggest that these mysterious mobile elements of the genome might play some kind of regulatory role, determining which genes are turned on and when this activation takes place. Around the same time as McClintock's ground breaking research, scientists Roy Britten and Eric Davidson hypothesised that TEs play a role in not only regulating gene expression, but also in generating different cell types and biological structures depending on where they insert themselves in the genome (Britten & Davidson, 1969). Even though all of a multicellular organism's cells have the same DNA, Britten and Davidson theorised that this could help explain why it contains so many different types of cells, tissues, and organs. As an example, consider your own body: Even though the bulk of cells in your body share the same DNA, you have dozens of different cell types. The early speculations of both McClintock and Britten and Davidson were largely dismissed by the scientific community. Only recently have biologists begun to entertain the possibility that this so-called "junk" DNA might not be junk after all.[3] Since then, they've been found in a wide range of eukaryotes. Recent genome sequencing initiatives have repeatedly revealed that TEs account for 50% of primate genomes, whereas coding DNA accounts for only 2%. Many studies suggest that finding of JUNK DNA are 540 million years old which is a long time for "junk" to survive if it serves

no purpose. Which further support the claim that junk DNA do have some function in body though it may not be known yet.[3] A large number of studies are to found out the actual role of JUNK DNA in human body which has revel that some part of the JUNK DNA is associate in development of diseases. Cancer is one such disease, Cancer is a condition where some of your body's cells become uncontrollable. They divide too rapidly, resulting in a deadly lump known as a tumor. Cancer is pretty common; you may know someone who has been diagnosed with it or have heard news reports about cancer patients. Different regions of the body might be affected by cancer. Breast cancer primarily affects women, with one in every eight women developing the disease at some point in their lives. Prostate cancer is just as common in men as breast cancer. So, how can spontaneous variation in junk DNA raise your chances of developing certain cancers? Junk DNA can have natural variation, too. Natural variation in junk DNA can increase your risk of cancer. So in following review we will visit what is the role of JUNK DNA in cancer.[4]

Blocking of telomerase elongation using BRD4 inhibitors

Long-term cell division necessitates the maintenance of telomere length. When telomeres get too short to safeguard chromosome ends, cellular senescence or apoptosis occurs.[5–7] This necessity for telomere length maintenance suggested that inhibiting telomere elongation could inhibit cancer cell proliferation in some circumstances.[8] Telomerase lengthens telomeres and maintains a telomere length balance that keeps them from becoming critically short.[9] Short telomere syndromes appear as bone marrow failure, immunodeficiency, enteropathy, pulmonary fibrosis, and emphysema in persons with insufficient telomerase or other telomere gene abnormalities.[10,11] Most cancer cells, on the other hand, increase the activity of telomerase.[12–14] Many malignancies have promoter mutations that boost the expression of the telomerase catalytic component TERT, according to recent research.[15,16] Germline mutations in the TERT promoter, or POT1, do, in fact, predispose to family melanoma, glioma, or CLL.[17–19] This shows that having lengthy telomeres could be a cancer risk factor.

For more than 25 years, telomerase inhibitors have been considered as potential cancer therapies.[8,20,21] BIBR1532 is a strong telomerase inhibitor in cell extracts and cell culture, but its solubility is poor, and it has yet to enter clinical trials. Imetelstat is an antisense chemical that binds to the intrinsic RNA template of telomerase to block it.[22–24] Imetelstat inhibits telomerase in vitro and causes telomere shortening in human cultured cells.[25,26]

However, phase II clinical trials were unsuccessful, and the manner of action in some cancers may be related to off-target effects.[27,28] With a better understanding of short and long telomere syndromes, as well as tumours that rely on telomerase, it's viable to rethink the idea of targeting telomere shortening in cancer with a more sophisticated approach.[29]

Several organisations discovered telomerase regulators by screening chemicals or genes that inhibit the telomerase enzyme.[30,31] Investigators explored an alternative strategy, looking for mechanisms that could prevent telomere lengthening without affecting telomerase activity or transcription. Shelterin proteins and post-translational modification play a role in telomere extension.[32–34] Scientists used an unbiased shRNA screen against kinases to find kinase pathways that potentially affect telomere length. BRD4 was identified as a novel positive regulator of telomere length in this screen. BRD4 is a member of the BET family of proteins with a bromodomain that binds to acetylated lysines. It also exhibits kinase and histone acetyl transferase activity. BRD4 is a multifunctional protein that regulates cell cycle, chromatin structure, and transcriptional control. BRD4 has never been linked to telomere length regulation before. Because multiple BRD4 inhibitors are now in clinical trials for cancer, it'll be crucial to figure out how they affect telomere length in order to figure out how they work and what adverse effects they might have.[35–39]

Enhanced chromatin engagement and transcription by the reaction of JUNK DNA with BRD4

Environmental signals and the coordinated activities of transcription factors and cofactors are combined by enhancers to shape gene expression programmes.[40–43] The capacity to annotate enhancers and their activity levels is based on the discovery of epigenomic characteristics such as an accumulation of the histone mark histone H3 lysine 4 monomethylation (H3K4me1), which, along with histone H3 lysine 27 acetylation (H3K27ac), distinguishes active enhancers.[44–47] Several transcriptional coregulators use BD modules to bind to acetylated histones, affecting chromatin accessibility and transcriptional activation at active enhancers.[48,49] The affinity and selectivity of BD interactions with acetylated lysines are typically low, but BD interactions with multiple acetylation sites in the histone tail can increase affinity and selectivity[50,51], implying that additional mechanisms regulating BD binding at enhancers are likely to have important implications for chromatin and gene regulation.

BET proteins use their tandem BDs to bind to histone acetylation sites (BD1 and BD2).[52] BRD4, a well-studied member of the BET family, regulates gene expression programmes by binding to acetylated histones and non-histone proteins at enhancers and promoters, which are important in inflammation and cancer formation.[53–55] BRD4 has been reported to interact with transcriptional regulators such as TWIST, p53, C/EBP, ERG, and NF-B, indicating that it has a function in enhancer and gene regulation.[56–59] BRD4 binding at enhancers is supported by BET family inhibitors, particularly JQ1, which have been shown to support super-enhancer development. BRD4 also impacts the synthesis of eRNAs by recruiting active positive elongation factor-b (P-TEFb) and histone chaperone activity, which affects RNA polymerase II (Pol II) elongation. Despite the fact that there are apparent linkages between BRD4 and gene regulation, the mechanisms behind BRD4's enhancer- and gene-specific targeting and activities are yet unknown. eRNAs have been linked to the control of gene expression in a variety of cell types and in response to various stimuli.[60] eRNAs have been connected to enhancer–promoter interaction interface control[61], chromatin remodelling, RNA Pol II pause release, and the recruitment of general cofactors such as cohesin, Mediator, and CBP in recent research.[62] Although eRNAs have the potential to add another layer of complexity to enhancer and gene control, the molecular basis for their action is still unknown. Researchers discovered that BDs serve as RNA-binding modules. In vitro and at active enhancers, we discovered that eRNAs cooperated with acetylated histone lysines to improve BRD4 binding and transcriptional activity. Our findings imply a feed-forward epigenetic process in which eRNAs promote an active enhancer landscape that affects proinflammatory gene expression via BRD4 connections.

In response to immunological signalling, BRD4 attaches to and acts at p53R273H, P309S-bound enhancers

Researchers wanted to see if BRD4 regulates enhancers that have recently been shown to be activated by p53R273H, P309S in response to chronic immunological signaling.[63] We detected stringent BRD4-binding peaks (n=21,528, P105) in human SW480 colon cancer cells expressing p53R273H,P309S (hereinafter mutp53) and treated with tumour necrosis factor alpha (TNF-) for 16 hours using genome-wide chromatin immunoprecipitation followed by sequencing (ChIP-seq). In response to chronic TNF signaling, comparisons of BRD4 peaks with previously reported mutp53, H3K4me1, and H3K27ac ChIP-seq data revealed colocalization of BRD4 binding peaks with mutp53 peak sites at intergenic locations, indicating enrichment of H3K4me1 and H3K27ac. Researchers discovered that

approximately one-third of the overall BRD4-binding sites (28 percent, n=5,949, P105) were at intergenic locations overlapping with active enhancers, and that over 80% (n=4,884, P105) of the BRD4-bound enhancers were also occupied by mutp53. De novo motif analysis also revealed that motifs recognised by NF-B/p65 and EWS-ERG fusion (ETS) were among the most highly enriched motifs to overlap with BRD4 and mutp53 peaks, which is consistent with our previous findings that NF-B recruits mutp53 to active enhancers in response to chronic immune signalling.[64] Researchers also used primer sets targeting downstream regulatory regions (amplicon B) and enhancer (amplicon A) regions of MMP9 and CCL2, which are among the enhancers that have previously been identified to be activated by mutp53 and NF-B in response to chronic TNF- signalling. In response to chronic TNF signalling, these experiments indicated simultaneous binding of mutp53 and BRD4 to the MMP9 and CCL2 enhancers, but not the control areas. We discovered that BRD4 and mutp53 formed physiological connections in SW480 cells before and after TNF-treatment using coimmunoprecipitation. BRD4 developed direct contacts with p53R273H and wild-type (WT) p53 using isolated proteins, which is similar to prior results of BRD4 interactions with WT p53. Following that, we used ChIP–qPCR to examine mutp53 contributions to BRD4 binding to enhancers in SW480 cells expressing doxycycline-inducible shRNAs against mutp53 after TNF treatment for 16 hours.

After TNF treatment, p53 shRNA significantly lowered mutp53 protein levels without changing BRD4 protein levels when compared to a non-targeting shRNA against LacZ (Ctrl). Mutp53 knockdown resulted in a significant loss of mutp53 binding at the MMP9 (66%) and CCL2 (72%) enhancer regions, as well as a significant reduction in BRD4 binding at the MMP9 (52%) and CCL2 (65%) enhancer areas. These findings show that mutp53 and BRD4 have functional interactions and that mutp53 is required for modulating BRD4 binding to active enhancers in response to chronic TNF signalling.[64]

BRD4 associates with RNAs produced in BRD4-occupied genomic areas

Researchers investigated whether BRD4 associates with RNAs by performing ultraviolet-cross-linked RNA immunoprecipitation (UV-RIP) in SW480 cells (TNF-) given the overlap between BRD4 enrichment and eRNA synthesis in response to chronic TNF- signalling and the previously described BRD4 association with RNA Pol II elongation complexes. Chronic TNF- administration had no effect on BRD4 mRNA or protein levels in this cell line. Despite the fact that the TFAP2A and MPP7 eRNAs were expressed at comparable or higher levels

than the MMP9, CCL2, CSF2, and TNFAIP3 eRNAs in TNF-treated cells, a BRD4 antibody specifically coimmunoprecipitated eRNAs produced from the MMP9, CCL2, CSF2, and TNFAIP3 enhancers, but not the TFAP2A and MPP7 enhancers. In response to chronic TNF signalling, BRD4 was found to bind with the MMP9 and CCL2 mRNAs, but not the TFAP2A and MPP7 mRNAs. BRD4's unique interaction with RNAs in response to chronic TNF signalling is consistent with the much greater TNF-induced RNA expression levels compared to uninduced RNA expression levels. BRD4-RNA connections were also consistent with BRD4 binding levels, which were shown to be considerably higher at the MMP9 and CCL2 enhancers and transcription start sites (TSSs) in response to chronic TNF signalling than at the TFAP2A and MPP7 enhancers and TSSs.

Through its tandem bromodomains, BRD4 interacts directly with eRNAs

Electrophoretic mobility shift assays were used to further study BRD4's direct binding to eRNAs (EMSAs). Purified BRD4-FL was incubated with a P-labelled MMP9 eRNA probe, which indicated a single significant BRD4–MMP9 eRNA complex. Quantification of the RNA EMSA with escalating doses of BRD4-FL revealed that at the maximum titration of BRD4-FL, BRD4-FL bound 80% of the MMP9 eRNA. Excess levels of unlabelled CCL2 eRNA competed specifically for BRD4 binding to the labelled MMP9 eRNA probe in competition EMSAs, but comparable concentrations of chilled MMP9 single-stranded DNA (ssDNA) were significantly less efficient, this shows that BRD4 preferentially binds to RNA in vitro. BRD4 deletion mutants and in vitro–transcribed MMP9 and CCL2 eRNAs were used to perform RNA pulldowns to determine the eRNA-interacting domains of BRD4. The MMP9 and CCL2 eRNAs were similarly bound by BRD4-FL and the naturally existing BRD4 isoform spanning amino acids 1–72248.[65] The BRD4 BDs, which are found inside the eRNA-interacting BRD4 (1–722) protein, were then evaluated for their contributions. Notably, researchers discovered a nearly complete loss of binding between BRD4 (BRD4 BD1/2) and the MMP9 and CCL2 eRNAs when BRD4 was devoid of both BDs. Similarly, radiolabeled MMP9 eRNA did not show a significant mobility shift with increasing BRD4 BD1/2 protein titrations in RNA EMSAs. In particular, BRD4 BD1/2 bound less than 5% of the tagged eRNA, but BRD4-FL bound 80 percent.

BRD4 binding to acetylated histone H3 and H4 peptides and histone octamers is enhanced by eRNAs

Researchers used in vitro binding tests with unmodified or acetylated H3 and H4 histone peptides to see how important eRNA-BRD4 interactions are in the modulation of BRD4 BD binding to acetylated histones. BRD4-FL, but not BRD4 BD1/2, bound more strongly to H3K27ac- and H4K16ac-modified peptides than unmodified peptides, as expected. In the presence of MMP9 eRNA, BRD4-FL binding to acetylated H3K27 and H4K16 peptides was enhanced. Furthermore, in the absence of BRD4 interactions with acetylated peptides, eRNAs did not promote BRD4 binding, as evidenced by the inability of eRNAs to support BRD4-FL binding to unmodified histone H3 and H4 peptides and the lack of an eRNA effect on BRD4 BD1/2 binding to acetylated H3K27 and H4K16 peptides. Researchers also looked at how eRNAs affected BRD4 binding to unacetylated or acetylated histone octamers by the histone acetyltransferase p300.[66] As expected, p300 efficiently acetylated the histone octamers, as seen by enhanced acetylation of H3K27 and H3K9, whereas in the absence of p300 or acetyl-CoA, little to no acetylation was detected. H3K27ac and H3K9ac antibodies were used to attach acetylated histone octamers to magnetic beads, which were then treated with BRD4 in the presence or absence of MMP9 eRNA. MMP9 eRNA increased BRD4-FL but not BRD4 BD1/2 binding to acetylated histone octamers, similar to the results of the histone peptide binding experiment. In the presence of the CCL2 eRNA, BRD4 binding to acetylated histone peptides and octamers was enhanced. Furthermore, eRNA titration experiments demonstrated that high molar ratios of MMP9 eRNA hindered BRD4 binding to acetylated histone octamers, whereas low molar ratios of MMP9 eRNA improved BRD4 binding to acetylated histone octamers.[66]

Drugs damaging Micro RNA and DNA in Breast cancer

Overview of Micro RNA

MicroRNAs (miRNAs) are small non-coding RNAs found in the human body that influence gene expression after transcription. Mature miRNAs are 18–25 nucleotide (nt) single-strand molecules generated by RNA polymerase II/III as lengthy primary transcripts with a hairpin structure, known as pri-miRNAs. The Microprocessor, a multi-protein complex that includes the RNase III enzyme DROSHA and its cofactor DGCR8, cleaves pri-miRNAs into 60 nt length molecules (pre-miRNAs) in the nucleus.[67] These molecules are recognised as pre-miRNAs by the nuclear export machinery, which is mostly made up of Exportin-5 and Ran-GTPase, and then exported to the cytoplasm for further processing. The second multi-domain

RNAase III enzyme DICER cleaves the dsRNA stem of pre-miRNAs asymmetrically into a short nucleotide duplex.

The transactivation-responsive RNA-binding protein (TRBP) regulates the assembly of the miRNA-induced silencing complex (miRISC) during this stage, preferring the interaction of DICER and Argonaute protein (AGO1, AGO2, AGO3, or AGO4). The mature miRNA (guide strand) is chosen by the miRISC complex, which subsequently directs the machinery to the target mRNA. The recognition of the "seed" region at the miRNA 5′ UTR and its complementary sequence on the 3′ UTR of the designated mRNA causes miRNA/mRNA interaction. Depending on the complimentary degree between the two sequences, the pairing results in either translational repression or transcript destruction.[68]

Following the discovery of the short RNA lin-4's role in Caenorhabditis elegans larval development in 1993, several researchers began to look into the regulatory potential of these tiny molecules. MiRNAs are recognised to play a role in practically every biological activity in mammals, including cancer. Several processes influence miRNA expression in cancer, including genomic abnormalities, epigenetic alterations, processing machinery failure, and transcription factor expression variations, to name a few. MiRNAs can function as tumour suppressors or oncogenes in cancer (oncomiR). Oncogenes are targeted by tumour suppressor miRNAs, which are frequently downregulated in cancer cells (e.g., miR-205 and miR-34 in breast cancer). Oncogenic miRNAs, on the other hand, target tumour suppressor genes and are frequently increased in tumour cells (for example, in breast cancer, miR-21, miR-155, and miR-221/222).[69]

MiRNA mimics and anti-miRs can be supplied using lipid carriers. For example, the miR-34-based therapeutic MRX34 (Mirna Therapeutics) uses the lipid carrier NOV40 to deliver miR-34 mimic sequence. MRX34 is the first miRNA-based cancer treatment that has been tested in a clinical trial. A phase I clinical trial enrolled patients with lymphoma, melanoma, multiple myeloma, liver, small cell lung, and renal cancer in 2013. Unfortunately, the experiment was halted in September 2016 due to severe and deadly immune-related adverse effects that occurred in some patients, despite the promising results obtained with partial responses in three patients and stable disease in 14 others. EnGeneIC Delivery Vehicle (EDV) nanocells (also known as TargomiRs) coated with epidermal growth factor receptor (EGFR)-specific antibodies are currently in a phase I trial to deliver miR-16 mimics in

patients with malignant pleural mesothelioma and non-small cell lung cancer (NSCLC), and preliminary results show that the treatment is well tolerated.[70]

Overview of Breast Cancer

Breast cancer is one of the most frequent cancers in women globally, and it is the leading cause of cancer-related death. Breast cancer has been described as a very varied illness based on histological and molecular traits, as well as responsiveness to therapy, by biological and genomic characterizations.[71] Breast tumours are classified clinically into the following subtypes based on the expression of three receptors that are typically tested by immunohistochemical assay: Triplenegative (ER–, PR–, HER2–) cancers are those that are oestrogen and progesterone receptor positive (ER+, PR+), human epidermal growth factor receptor positive (HER2+), and oestrogen and progesterone receptor negative (ER–, PR–, HER2–).[72,73] In addition to the histological grade, clinical stage, patient's age, and menopausal state, this categorization provides useful clinical information, mostly for selecting the first-line treatment. High-throughput technologies, such as microarray-based transcriptome analysis, have opened up new avenues for learning about breast cancer biology. Breast tumours were divided into five intrinsic molecular subgroups based on gene expression profiles: hormone receptor positive luminal A and luminal B, HER2-enriched, basal-like, and normal-like. The occurrence, prognosis, and therapeutic responsiveness of these subgroups varied. Luminal B cancers (ER+, PR+, HER2–, Ki67+) have a higher clinical grade than luminal A tumours (ER+, PR+, HER2–, Ki67–), and some of them express the HER2 receptor. The HER2-enriched (HER2+, ER–, PR–) subgroup is characterised by high grade and node positivity, whereas the basal-like (HER2–, ER–, PR–) subgroup is characterised by triple-negative breast tumours (TNBCs) and frequently demonstrates germinal and sporadic BRCA1 mutations.[74]

The claudin-low subtype, derived primarily from the TNBC subgroup and characterised by stem cell-like characteristics, is a newly described intrinsic subtype. Lehmann and colleagues identified six subtypes with unique gene expression patterns and treatment responses to provide a more detailed classification based on the molecular portrait of TNBCs in 2011. Immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtypes. The BL1 and BL2 subtypes, which are selectively sensitive to cisplatin, express a high level of cell cycle and DNA damage response genes. The M and MSL subtypes, which respond to PI3K/mTOR and abl/src inhibitors, have an

abundance of epithelial-to-mesenchymal transition and growth factor pathways. The LAR subtype is responsive to bicalutamide, which is characterised by androgen receptor (AR) signalling and shorter relapse-free survival (an AR antagonist). Next-generation sequencing (NGS) has enhanced the molecular characterisation of breast carcinomas dramatically in recent years, providing information on gene mutations, DNA copy number variations, DNA methylation, and miRNA expression patterns.

Mechanisms of DNA Repair

One of the hallmarks of cancer is genomic instability, which is well-known. Breast cancer cells have been shown in numerous studies to have faulty DNA damage response (DDR) pathways. When cells are damaged by DNA, they correct the errors and continue to multiply; alternatively, the damage can result in mutations or chromosomal rearrangements, which can lead to cancer. The DNA damage response (DDR) is a complex system that is shaped by the activities of DNA damage signal transduction, DNA repair mechanisms, cell cycle checkpoints, and apoptosis signalling pathways.[75] DDR controls DNA repair by triggering the following molecular processes: damage site recognition, recruitment of repair components, and DNA lesion repair. DNA damage sensors, signal transducers, and effectors make up the DDR machinery. DDR can repair DNA damage through a variety of ways. Two processes are specifically dedicated to remove damaged and changed nucleotides: (1) Nucleotide Excision Repair (NER), which eliminates single nucleotides via methylation, alkylation, deamination, or oxidation, and (2) Base Excision Repair (BER), which removes helix-distorting and transcription-blocking lesions (i.e. UVinduced pyrimidine dimers). Another DDR process helps to detect erroneous nucleotide insertions or deletions during DNA synthesis, which can cause microsatellite instability and cancer. These mistakes produce base mismatches in the canonical DNA sequence, resulting in DNA helix distortion. MSH2 and MLH1, which form heterodimers with MSH3 or MSH6 and MLH3, PMS2, or PMS1, respectively, are part of the Mismatch Repair Machinery (MMR).[76]

Other types of DNA damage, such as Double-Strand Breaks (DSBs) or Single-Strand Breaks (SSBs), can be caused by a variety of environmental factors (SSBs). SSBs and modified bases are the most prevalent DNA damage, with 20,000 events per cell occurring per day, but they are usually repaired by the BER mechanism. Instead, DSBs cause the MRE11–RAD50–NBS1 (MRN) complex to be recruited, which activates the serine/threonine-specific kinases ATM, allowing them to autophosphorylate (pATM) and phosphorylate Ser139 of histone

H2AX (H2AX) in response to DNA damage signals. H2AX binds more pATM molecules and DDR proteins to the DSB site, such as p53-binding protein 1 (53BP1), to form nuclear foci. ATM kinase increases the phosphorylation of several proteins in response to DSBs, including Chk2 kinase, one of ATM's most critical effectors. ATR, on the other hand, recruits Chk1 kinase in response to halted replication forks and UV-induced SSBs.[77] When damage repair is ineffective, ATM and ATR cause phosphorylation of numerous proteins, which activates downstream DNA repair pathways and causes cell cycle arrest, death, or senescence.

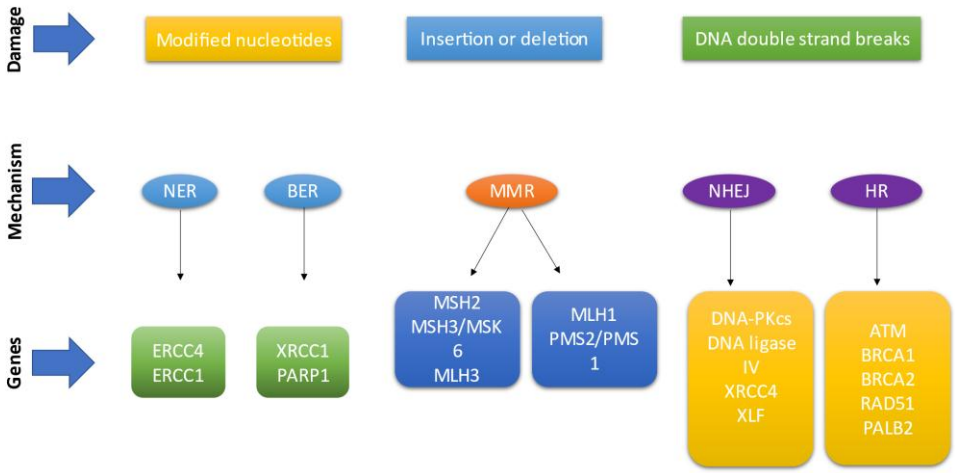

Figure 1: Depicts a schematic overview of DNA damage repair methods as well as some key miRNAs involved in DNA repair gene regulation. As a result, miRNAs are a major regulator of DNA repair pathways and a fresh source for exploiting DDR gene/miRNA interactions in clinical settings as diagnostics and therapeutic tools.

DRUGS THAT AFFECT DNA IN BREAST CANCER THERAPY

Surgery, radiation, chemotherapy, hormone therapy, and biological targeted therapy are all options for treating breast cancer. Patients with hormone receptor positive (ER+, PR+) cancers are treated with hormone treatment (tamoxifen and aromatase inhibitors), whereas those with HER2+ malignancies are treated with anti-HER2 targeted therapy (trastuzumab and pertuzumab). As a result, the clinical management of TNBCs poses the greatest challenge, owing to a paucity of specific therapies. Indeed, cytotoxic chemotherapy is still the standard treatment for malignant cancers. Luminal A tumours are less responsive to

chemotherapy, while luminal B tumours are more responsive than luminal A but less so than HER2-enriched and basal-like tumours, which have a greater response rate.

Radiotherapy and chemotherapy are commonly employed as first-line medications in combination with hormone and target therapies in the treatment of breast cancer because they cause DNA damage. Ionizing radiation (IR), anthracyclines, platinum compounds, and taxanes are all known to cause DSBs and SSBs; nevertheless, the efficacy of today's DNA-damaging medications is linked to cancer cells' ability to resolve and repair DNA lesions.

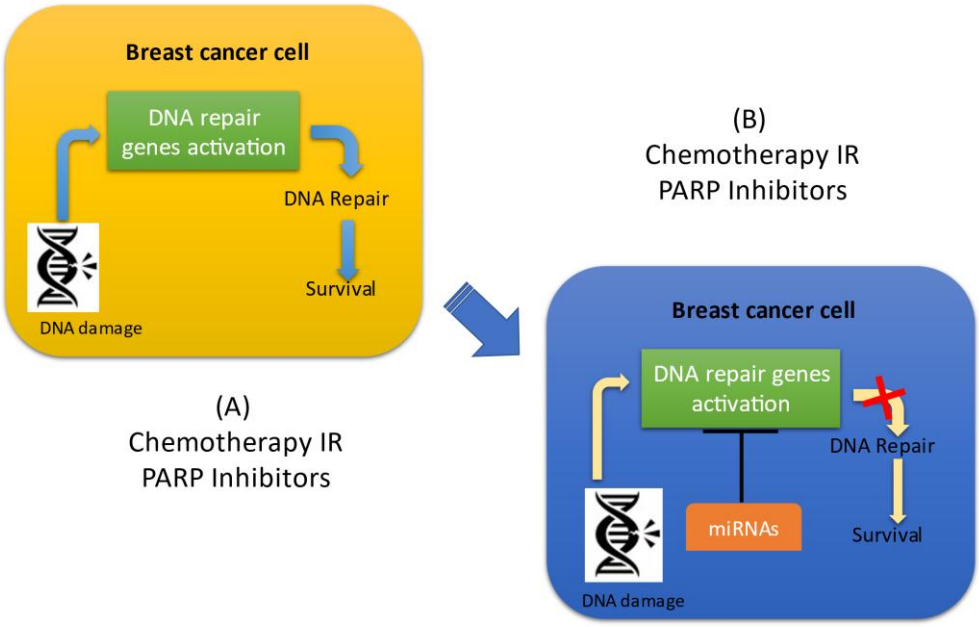

Figure 2: In comparison to normal cells, cancer cells proliferate rapidly, which makes them more vulnerable to DNA damage during the S phase of the cell cycle. The biggest issue with radiotherapy and chemotherapy, however, is the development of acquired resistance during medication administration. The activation of multi staged mechanisms that increase tumour cell death is induced by radiotherapy treatment, which is based on the injection of a certain amount of energy. DSBs, in particular, cause chromosomal changes and influence cell division, resulting in cell death or mutation.[78]

Ionizing radiation (IR), such as X-rays, can cause further cell damage by breaking DNA directly or indirectly by creating free radicals. Tumours that are exposed to a high total dose of radiation might develop radio resistance, which can lead to treatment failure. The development of radio resistance has been linked to changes in the expression of cell cycle

components and DDR effectors, such as cyclin D1 overexpression and constitutive activation of DNA-PK and KT, respectively.[79]

Chemotherapy is the most commonly used cancer treatment. Direct cytotoxicity, activation of the host immunological response, inhibition of cell proliferation, and induction of apoptosis are all ways that chemotherapeutic drugs cause tumour cell death. DNA damage is the first event detected by the cellular stress response machinery after cytotoxic chemicals are administered, causing the activation of effector systems such as apoptosis. Unfortunately, resistance to chemotherapy can manifest itself in a variety of ways, including DNA repair, cell cycle regulation, and apoptosis avoidance.

DNA REPAIR GENES AND RADIOCHEMOTHERAPY RESPONSIVENESS ARE CONTROLLED BY MICRORNAS

Cancer development and progression are characterised by changes in DNA repair pathways and miRNA expression. Genotoxic chemicals, which cause DNA damage, are routinely utilised for radio- and chemotherapeutic therapies in breast cancer, according to this research. MiRNA modulation, either up or down, is frequently implicated in the regulation of DNA repair pathways, and it is now known that miRNAs can regulate drug response.[80] As a result, changes in miRNA expression involved in DDR pathways play a key role in radio- and chemotherapeutic response. Our team recently discovered that miR-302b expression in breast cancer cell lines causes cisplatin sensitivity, resulting in decreased cell survival and proliferation in response to the drug.

MiR302b directly targets E2F1, a master regulator of the G1/S transition. Furthermore, through negatively regulating E2F1, this miRNA indirectly downregulates ATM, the primary cellular sensor of DNA damage, impairing cell-cycle progression after cisplatin therapy. As a result, miR-302b reduces the ability of breast cancer cells to repair damaged DNA after exposure to cisplatin, increasing apoptosis. Accordingly, another group has demonstrated that the miR-302 family can sensitise breast cancer cells to radiotherapy; in particular, Liang et al. demonstrated that decreased expression of miR-302a induces radiotherapy resistance and reintroduction of miR-302a expression enhances radiotherapy sensitivity in in vitro and in vivo breast cancer models, abrogating AKT1 and RAD52 expression.[81]

In vitro and in vivo models, Gasparini et al. discovered that miR-155 overexpression lowered RAD51 levels in human breast cancer cells, which impacts the response to IR and decreases the efficiency of HR repair, boosting IR sensitivity.

Furthermore, high levels of miR-155 and low expression of RAD51 were found to be associated with a superior overall survival in a group of TNBC patients. As a result, miR-155 expression can be used as a predictive biomarker to identify TNBC patients who are likely to respond to an IR-based treatment.[82]

Overexpression of the oncomiR miR-21 in cancers has been widely demonstrated, with implications for cell cycle, DNA damage repair, apoptosis, autophagy, and hypoxia of cancer cells after irradiation. Indeed, miR-21 influences cell cycle progression by inducing DNA damage in the G2 checkpoint, and miRNA suppression (via anti-miR-21 injection) reduced the G2/M barrier and caused apoptosis in breast cancer cells after radiation treatment.

In preclinical breast cancer models, overexpression of the oncosuppressive miRNA miR-205 enhances the responsiveness to tyrosine kinase inhibitors, lapatinib and gefitinib. It was recently shown that increased expression of miR-205 makes breast cancer cells more sensitive to radiation through influencing ZEB1 and altering DNA repair. Indeed, miR-205 is a direct target of Ubc13, a homologous recombination protein. Furthermore, the authors revealed that nanoliposome administration of miR-205 mimics has a therapeutic impact in a xenograft model, sensitising the tumour to radiation.[83]

MiR-18a is increased in breast cancer cell lines and tissues, and its ectopic expression downregulates ATM, which is noteworthy. This event lowered the ability of breast cancer cells to repair DNA damage, reduced HR efficiency, and made them more sensitive to radiation treatment. Wip1 is a DNA damage signalling pathway regulator, specifically inhibiting the phosphorylation of ATM, Chk1, Chk2, p53, and other DNA repair proteins. MiR-16 targets Wip1, which affects DNA repair and makes breast cancer cells more susceptible to doxorubicin therapy, according to Zhang et al.[83]

Table 1: Finally, a number of research have focused on miRNAs that target the BRCA genes in breast cancer. BRCA1 and BRCA2 are tumour suppressor genes that play a role in the HR mechanism, which is involved in the repair of DNA double strand breaks (DSBs). For example, miR-218 targets BRCA1 directly, and its restored expression in cisplatin-resistant breast cancer cell lines sensitises the cells to the medication, reducing DNA damage.

MicroRNA expression	Gene target	Drug response
miR-638 overexpression	BRCA1	UV and Cisplatin sensitivity
miR-218 overexpression	BRCA1	Cisplatin sensitivity
miR-96 overexpression	REV1 and RAD51	Cisplatin sensitivity
miR-16 overexpression	Wip1	Doxorubicin sensitivity
miR-18a overexpression	ATM	IR sensitivity
miR-205 overexpression	Ubc 13	IR sensitivity
miR-21 downregulation	G2/M block	IR sensitivity
miR-155 overexpression	RAD51	IR sensitivity
miR-320a overexpression	AKT1 and RAD52	IR sensitivity
miR-302b overexpression	E2F1 and ATM	Cisplatin sensitivity

Table 1 lists the miRNAs that regulate DNA repair genes in breast cancer and are implicated in chemo- and radio-responsiveness.

DNA REPAIR GENES AND PARP INHIBITOR RESPONSE ARE CONTROLLED BY MICRORNAS

PARP inhibitors, as previously said, are one of the most cutting-edge approaches in the development of anti-breast cancer medicines. However, it is yet to be determined if miRNAs play a role in PARP inhibitor sensitivity. Indeed, the role of miRNAs in the modulation of PARP inhibitor responsiveness has received little attention. The key findings about this regulation in breast cancer are presented below. Moskwa et al. reported in 2011 that breast cancer cells overexpressing miR182 are more susceptible to IR and PARP inhibitors due to BRCA1 targeting and DNA repair impairment. Mice implanted with breast cancer cells overexpressing miR-182 demonstrated reduced tumour development when treated with the PARP inhibitor olaparib, confirming the findings in in vivo models. [84] Furthermore, it has been established that CHEK2, another HR-related gene, is a direct target of miR-182-5p. This modulation improves the susceptibility of breast cancer cells to a PARP inhibitor. MiR-107, miR-222, and miR-103 regulate the DDR and sensitise tumour cells to PARP inhibitors in breast cancer cell lines, according to Neijenhuis et al. and Huang et al., by targeting RAD51 and inhibiting HR.[85,86]

It's also known that miR96 targets RAD51 and REV1, and that overexpression of this miRNA in breast cancer in vitro models improves PARP inhibitor sensitivity. TGF has also been shown to regulate DNA repair genes as well as PARP inhibitor response in this scenario. TGF-induced sensitivity to PARP inhibition is mediated by two TGF-targeted DNA-repair genes, ATM and BRCA1, both regulated by miR-181, and MSH2, which is regulated by miR-21. The role of miR-664b-5p has recently been studied. This miRNA is a tumour suppressor that is increased in response to PARP inhibitors and chemotherapy. As a result, miR-664b5p plays a key role in the regulation of PARP inhibitors, which increases chemosensitivity by targeting CCNE2 in BRCA1-negative TNBC.[87]

Epigenetic regulation of Paediatric cancer

Epigenetics is derived from the Greek word epigenetics, which meaning "over and above" (epi) the genome. Conrad Waddington coined the phrase in 1939 to describe the method by which a feature acquired in a population in response to an environmental stimulation is passed down through the generations.[88] His research on fruit fly embryos revealed that changes in environmental temperature or chemical stimuli can influence the thorax and wing architecture of some fruit flies.[89] He took flies from their parent population that had developed these diverse phenotypes and bred them for 20 generations to test if the new features were passed down to the progeny. After a few generations, he discovered that the new characteristic was robustly inherited even without the environmental stimulus. He coined the term "genetic absorption" to describe this process, claiming that it may be because the genotype was already present in the population before selection began, and all that was needed was to provide the environmental stimuli.[89]

Because the features that fruit flies acquired as a result of environmental stress were already present in the population, he was correct in his conclusions. It's also unlikely that the fruit fly population's developmental adaptability is the consequence of random genetic changes. This is due to the fact that each mutation is rare within a population and takes several generations to propagate across entire populations. Conrad Waddington rejected the Neo-Darwinist explanation for this occurrence (random mutation causes genetic differences). He coined the term "epigenetics," which refers to inheritance in addition to (epi) conventional genetics without additional mutations.

Since then, we've learned a lot more about epigenetics. Chemical changes in a single gene without a change in nucleotide sequence have been shown to alter gene activity.

The addition of chemical substances to genes to influence their expression is referred to as epigenetic (meaning above the DNA). Epigenetic alteration patterns can be passed down across generations, vary between cells or individuals, and are impacted by environmental factors. Chromosomal DNA is condensed by tightly coiling several times around the histone protein family, and the resulting DNA-protein complex is referred to as chromatin.[90]

A nucleosome is an octameric structure with a diameter of roughly 11 nm. H5/H1 is a histone linker that binds to the nucleosome around the DNA entry and exit sites, thereby securing the DNA in place. The DNA wrapped around these histones is 20 base pairs long, resulting in two full DNA twists around each nucleosome. H5/H1 histones also bind to the "linker DNA" (about 36 base pairs) that joins and holds adjacent nucleosomes together. A chromosome is made up of hundreds of nucleosomes joined by linker DNA and closely packed together. The lengthy chains of nucleosomes on the chromosome generate a beadlike look on a DNA string when seen under a microscope.[91]

Access to this closely packed DNA is required by cell transcriptional machinery, which is controlled by alterations to both the DNA and histone tails. These changes affect the overall structure of chromatin, making it either decondensed (euchromatin) or compacted (euchromatin) (heterochromatin). Euchromatin that is decondensed, or loosely packed, allows transcription factors access and is transcriptionally active, whereas heterochromatin that is densely packed is inaccessible and transcriptionally mute. Gene transcription and regulation are affected by epigenetic changes or tags in the DNA, which change the structure and accessibility of chromatin.

Epigenetic Modifications

(1) DNA Modifications

There are two types of epigenetic modifications: those that occur on DNA and those that occur on histone proteins. **(figure)** The addition of a methyl group to the 5-carbon of the cytosine ring, resulting in 5-methylcytosine, is one of the most common DNA modifications. Cytosine methylation is most common in the mammalian genome at CpG sites, which are DNA sequences where cytosine is followed by guanine in the 5′ -3′ direction. CpG methylation is a critical component in genomic imprinting, tissue-specific gene expression throughout development, differentiation, X chromosome inactivation, and suppression of transcription repeat elements and transposons.[92,93] CpG sites in CpG islands (CGIs), which are regions of the genome with a large number of CpG dinucleotide sequences, are

unmethylated. CGI areas can be found in around 70% of gene promoters, including all housekeeping genes and transcription start sites that have been characterised. The majority of CGIs are unmethylated in order to promote transcription, although a tiny fraction of them gain methylation during normal development. For example, during dosage adjustment, X-chromosome inactivation silences most genes on one X chromosome (Xi) in each female cell. The methylation of CGIs present at the promoters of genes silenced after X-chromosome inactivation is increased on the Xi chromosome. Recent genome-wide DNA methylation studies have suggested that methylation may control transcription in a beneficial way. The presence of methylation in the gene bodies does not prevent transcription elongation and is associated with active transcription.

The function of methylation in the proximity of transcription start sites, where it hinders transcription initiation, is paradoxical. Harris et al. found that DNA methylation recruits a protein complex on chromatin that increases the transcription of genes that are already modestly transcribed but has no effect on transcriptionally quiet transposable elements in Arabidopsis thaliana.[94]

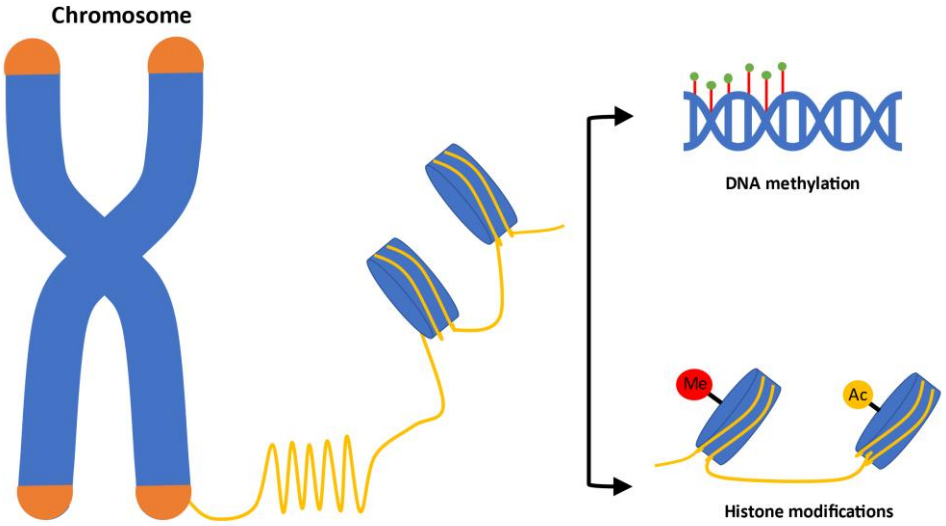

Figure 3: This shows that DNA methylation may play a dual role in regulating gene expression by balancing transcriptional activation and repression. DNA methyltransferases (DNMTs) transfer a methyl group (CH3) from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA, catalysing DNA methylation. Dnmt1, Dnmt3a, and Dnmt3b are the three varieties of Dnmts found in mammalian tissues. They have a big N-terminal regulatory domain and

a C-terminal catalytic domain in common, but they have different roles and expression patterns.[95,96]

(2) Histone Protein Modifications

Phosphorylation, methylation, acetylation, ubiquitination, and SUMOylation are some of the modifications made to histone proteins. The majority of these alterations take place at the amino and carboxy-terminal tails of histones, which extend into the region around nucleosomes. Some locations within histones' globular core domains are also modified, and they play an important role in chromatin dynamics and transcriptional control. Dot1 (disruptor of telomeric silencing-1) methyltransferases methylate lysine 79 (H3K79) in the globular domain of histone 3 (H3K79). H3K79 methylation has been found in active genes' transcribed areas, and it is known to influence telomerase silencing, transcriptional elongation, cell cycle, and embryonic development.[97]

Histone alterations can affect chromatin folding, allowing DNA to access replication machinery, or being identified by chromatin remodelling enzymes with histone mark-specific binding domains. The chromatin remodelling enzymes are multi-subunit complexes with an ATPase domain that hydrolyze ATP to cause a variety of structural changes in chromatin by shifting nucleosome locations and breaking DNA-histone interactions.[98,99] Based on their biochemical features and distinctive domains within or next to the ATPase domain, these enzymes can be classified into four subfamilies. SWI/SNF (SWItching Defective/Sucrose NonFermenting), ISWI (Imitation SWItch), CHD (Chromodomain, Helicase, DNA Binding), and INO80 are chromatin remodeler families (INOsitol requiring 80). All remodelling complexes' ATPase subunits have a DExx and a HELICc domain separated by a linker, although the domains are distinct.

The SWI/SNF family was named after the SWI/SNF complex, which was discovered in yeast as the first ATP-dependent chromatin remodelling enzyme. This family is important for ejecting nucleosomes from various loci in an ATP-dependent manner, causing nucleosome structure to change. The SWI/SNF complexes are not involved in chromatin assembly. The extra spaces present in their ATPase subunit incorporates a QLQ space that is significant for protein cooperation, a helicase-SANT-related (HSA) space that associates with actin-related proteins (ARPs) present in numerous chromatin remodelers and manages ATPase movement, a BRK area that shows grouping comparability to the glycine-tyrosine-phenylalanine (GYF) area that ties with proline-rich groupings to advance protein associations and different

bromodomains, a developmental monitored protein collaboration module that perceives acetylated lysine build-ups on histones N terminal tails. The ISWI family has an ATPase domain that is similar to SWI/SNF but incorporates SANT and SLIDE (SANT-like ISWI) domains instead of the bromodomain at the C terminus of the ATPase subunit.[100] The SANT and SLIDE domains, which are conserved, mobilise nucleosomes and cause nucleosome spacing. The location of nucleosomes relative to DNA, as well as nucleosome spacing, influence DNA accessibility to regulatory factors and chromatin rearrangement, all of which are important in the biological activities inherent in DNA. CHD (chromodomain, helicase DNA-binding) family remodelers were first isolated from Xenopus laevis and have transcription-repressive or transcription-promoting actions.[101] In the SWI/SNF complex, all CHD proteins have two tandem chromatin organisation modifier (chromo) domains in the HSA domain and two Sucrose NonFermentable2 (SNF2)-like ATP-dependent helicase domains. The latter increases nucleosome mobilisation, whereas the chromodomains bind to changed histones specifically. CHDs are classified into two types: CHDs and NuRD (nucleosome remodelling and deacetylases). CHDs induce transcription, whereas NuRD, which is both an ATPase and a histone deacetylase (HDAC), operates as a transcriptional repressor.[102]

CONCLUSION

Long-term synaptic plasticity and memory are influenced by the transcription factor cyclic AMP response element-binding protein 2 (CREB2). Eric Kandel and colleagues from The Rockefeller University screened for neuronal piRNAs in the central nervous system of the marine snail Aplysia californica (Aplysia) and discovered 372 different piRNA clusters to investigate the role of piRNAs in memory store control. After treatment with serotonin or 5-hydroxytryptamine, a critical learning and memory specific monoamine neurotransmitter, neuronal piRNA expression levels were examined in Aplysia CNS cells, and an increase of a subset of the selected piRNAs, such as aca-piR-4 and aca-piR-15, was discovered. Because piRNAs and Piwi proteins are known to play a role in epigenetic regulation by DNA methylation in germline cells, the authors investigated the role of DNA methylation in CREB2 regulation by treating neurons with RG108, an ApDNMT inhibitor, and found that CREB2 levels were significantly increased. The proximal promoter CpG island methylation was elevated in neurons following serotonin exposure, but not the distal CpG island, and remained unmethylated in the presence of RG108, showing that CREB2 promotor methylation occurs in response to serotonin-induced synaptic plasticity. Telomere extension

is aided by shelterin proteins and post-translational modifications. To uncover kinase pathways that could alter telomere length, scientists conducted an unbiased shRNA screen against kinases. These findings revealed simultaneous binding of mutp53 and BRD4 to the MMP9 and CCL2 enhancers in response to chronic TNF signalling, but not to the control regions. Nonetheless, as our understanding of genomes improves, genomes appear to be significantly more elegant than previously appreciated or projected by some Darwinists. Design visible in genomes can be handled by Darwinism in the same manner that other evidence of design can be accepted by referring to it as 'apparent' design rather than true design. Histone 3 lysine 9 trimethylation (H3K9me3) is an epigenetic change to the DNA packing protein Histone H3, which is known to be involved in a variety of biological processes via influencing gene expression. Transposable elements are key parts of the eukaryotic genome, and can change the cell's genetic identity by causing mutations or duplication of similar genetic material. Through germ cells, these changes will be handed on to future generations. Through identifying and silencing a wide range of transposable components in germline cells, a team led by Alexei Aravin of the California Institute of Technology investigated the critical role of piRNAs in ensuring fertility and the authentic transmission of genetic material to future generations. However, greater levels of H3K9me3 on LINE families were identified in germ cells. These components are controlled in germ cells by an additional mechanism that is less active or non-existent in somatic cells. The H3K9me3 state in germ cells was lower in Miwi2 mutant animals than in somatic cells, showing that the piRNA pathway is necessary for the higher H3K9me3 status on these LINEs expression in germ cells. Furthermore, finding piRNAs on full-length LINEs in the genome indicated repression of full-length LINEs but not fragments, implying that a flaw in the piRNA pathway only affects full-length copies of transposable elements.

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