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REGULATION OF MICRO-RNA IN CANCER

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Abstract:

Cancer is a dreadful disease of mankind, the treatment for cancer is not revealed as per expectation. The illuminating way come out with understanding and grab the molecular alteration in cell. Therefore, miRNAs is a novel notation for procurement of cancer. MicroRNAs are small, highly conserved non-coding RNA molecules involved in the regulation of gene expression. MicroRNAs are transcribed by RNA polymerases II and III which forms precursors that undergoes series of cleavage to form mature microRNA. There are two types of biogenesis pathways, one nuclear and one cytoplasmic. However there are some alternative biogenesis pathways exist that differ from conventional pathway in the number of cleavage events and enzymes responsible. The mechanism of sorting of microRNA precursors to the different pathways is unclear but it can be determined by the site of origin, its sequence and thermodynamic stability. The regulatory functions of microRNAs are able through the RNA-induced silencing complex (RISC). The regulation level of miRNAs in cell i.e. up regulation and down regulation, leads to cancer. In this review, highlighted the role of miRNAs in physiological way and explain the molecular mechanism involved in development of cancer.

Key words:*MicroRNA, Cancer, RNA-induced silencing complex (RISC), RNA polymerases II and III.*

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INTRODUCTION:

The body is composed of trillions of living cells. Normal body cells grow, multiply, and die in an orderly fashion. During the early years of a human's life normal cells divide faster to allow the person to growth. When the person becomes an adult, most cells separate only to repair injuries or to replace dying cells.

Cancer causes millions of death worldwide and there is a continued search ongoing for new effective therapies, as well as biomarkers to assess the likelihood response to these therapies. Due to some technologies and current advances in understanding the molecular basis of tumours have developed some interest in the development of new, rationally designed, targeted agents. The major obstacle in the treatment of cancer is drug resistance which limits the potency of both conventional chemotherapeutic and novel biological agents [1].

In U.S., cancer is the second deadliest disease after cardiovascular disease and in UK it is the leading cause of death [2,3]. Cancer incidence appears much lower in many Third World countries, most likely because of the higher death rates due to infectious disease or injury. The cancer is expected to rise in the Eastern Mediterranean region, cancer incidence is increasing day by day and it is expected to increase by 100 to 180% in the next 15 years [4].
Cancer epidemiology closely mirrors the risk factor spread in various countries. Liver cancer is the main cancer observed in China and neighboring countries and it is rare in the West region, mostly due to the endemic presence of aflatoxin and hepatitis B in that population. Similarly, as the tobacco smoking is becoming more common cause of lung cancer in various Third World countries.

In 2010, over 12 million cancer cases found and 7.6 million cancer deaths occurred worldwide [5]. Survival rates in cancer increased due to improvements in the detection and treatment of it. Those patients diagnosed with metastatic cancer can survive years to decades beyond their early diagnosis [6]. For many patients, the first sign of cancer is not only associated with pain but also affecting the quality of life and functional status [7, 8]. Cancer pain is the most life threatening stage during the course of the disease. Cancer pain tend to increase with its severity. So patients with metastatic or advanced-stage cancer will experience significant cancer-induced pain [9-11].

Factor involved in induction of cancer:

The cytoskeleton is involved in many aspects of cellular function such as phagocytosis, muscle contraction, cell movement and mitosis. Any alteration in its structure affects cell physiology in many ways. It is recommended that there is a link between cell shape changes, cytoskeleton dynamics, and alterations of gene expression. By interacting with members of transduction pathways the cytoskeleton may control the localization of signaling molecules and thus regulate gene expression [12]. The cytoplasmic microtubules is a major element of the cytoskeleton which may be involved in intracellular signaling. Due to their dynamic instability, microtubules are subjected to constant remodeling. Agents that alter cell cycle progression, microtubule assembly and changes in the cytoskeleton induce a range of cellular responses [13]. Some of these agents stimulate mitogen activated protein kinases [14-16] and modulate gene expression [cyclooxygenase-2 [17], tumor necrosis factor-a, interleukin-1, CHUK, etc. [18]. Previously it is observed that drug-mediated inhibition of microtubule polymerization is accompanied with the up-regulation of the nuclear cmyc gene. The c-myc proto-oncogene plays a critical role in basal cell growth and deregulation of this gene is involved in the development of a variety of tumors [19]. The product of the c-myc gene is a nuclear phosphoprotein that has been concerned in the regulation of cell proliferation, cell differentiation and apoptosis.[20, 21]. Temporary induction of a low level of c-myc mRNA follows the growth activation of all the inactive untransformed cells [22]. Due to increase in expression cells enters into S phase of cell division. For heterodimerization the c-Myc protein contains multifunctional regions i.e. N-terminal transactivation domain and C-terminal domain. These data supported the model of c-Myc functioning as a transcription factor whose activity can be regulated by its protein binding partners. There are multiple cis elements located on the enhancer influencing the transcription of c-myc, some of which have been shown to bind nuclear proteins [23]. Among them, NFkB has two binding sites on the c-myc promoter [24]. NFkB was known as a mediator of activation of the k-light chain gene in B cells [25]. NFkB is a heterodimer which consist of two subunits i.e. p50 and p65, these subunits are capable of homodimerizing and binding to specific targets on its own. For regulating cellular localization NFkB forms complex with the inhibitory protein IkB in cytoplasm. IkB contains ankyrin repeats that are thought to form binding sites for both integral membrane proteins and tubulin [26].

Micro-RNA:

In recent years, many new small functional RNAs are identified. RNA is usually thought to be a messenger RNA which serves as the template for translation of genes into proteins. Non-coding RNA molecules are transcribed from a DNA sequence but not translated into protein. The encoding DNA sequence is often referred to as an RNA gene. There are mainly three types of functional RNA genes in the human genome i.e. m-RNA, transfer RNA (tRNA), ribosomal RNA (rRNA) and various other small non-coding RNAs. MicroRNAs (miRNAs) encodes several hundred genes in our genome. Precursors of these miRNA molecules form structures of double-stranded RNA that can activate the RNA interference machinery. The gene expression is down regulated by m-RNA either by degradation of messenger RNA through RNA interface pathway or by inhibition of protein translation.

MicroRNAs (miRNA) are small sized, noncoding RNA, molecules having 19–24 nucleotides. At the post-transcriptional level these RNA down regulate protein expression, by specifically binding to the 30-untranslational region (30-UTR) of mRNAs, and thus preventing their translation and promote their degradation [27,28]. Particularly, a single miRNA can regulates number of target genes simultaneously, almost all of the protein coding genes are regulated by miRNA [29,30]. It is demonstrated that mi-RNAs play a key role in the development, function and maintenance of tissues and cells in various organisms [31]. From various studies it is shown that miRNAs function as tumor suppressors or oncogenes to modulate multiple oncogenic cellular processes i.e. apoptosis, invasion, cell proliferation and metastasis [32].

The first mi-RNA was discovered by Victor Ambros and colleagues Rosalind Lee and Rhonda Feinbaum in 1993. A genetic screen in the roundworm *Caenorhabditiselegans*, a millimeter long animal used as a model organism in biological research [33]. One of the gene termed as lin-4 did not encode a protein but instead a novel 22-nucleotide small RNA was observed. Seven years later, Reinhart et al. discovered a second 22-nucleotidesmall RNA of this type i.e. let-7 a gene also involved in C. elegans developmental timing [34]. Small regulatory RNAs, lin-7 and let-4 soon became very exciting for two reasons. Firstly, some homologs of the gene let-7 were identified in other animals including humans [35]. The conservation of let-7 across species suggested an important and fundamental biological role for this small RNA. Secondly, the mechanism of RNA interference (RNAi) was discovered at the same time, and it became clear that miRNA and RNAi pathways were linked to each other and shared common components. There are more than 100 additional small regulatory RNAs similar to lin-4 and let-7 were identified in worms, in fruit fly Drosophila, and in humans. These small non-coding RNAs were then named as micro RNAs (miRNAs) [36-38].

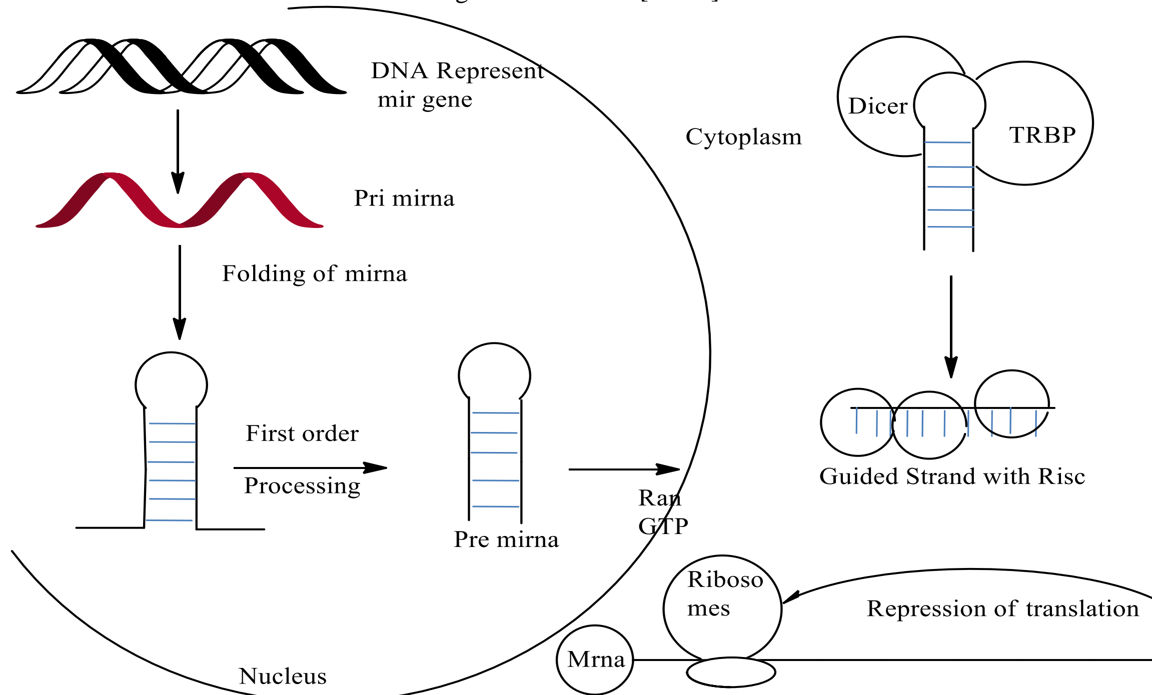

Fig.1: Physiological role of mi-rna

Regulation of MIRNAS:

Up-regulation and down-regulation:

Overall regulation of gene expression includes a wide range of mechanisms that are used by cells due to which there is increase or decrease in the production of specific gene products, and is termed gene regulation. Mechanism of gene expression is widely used in biological phenomenon. Any step of gene expression can be modulated i.e. from transcriptional initiation to RNA processing and to the post-translational modification of a protein.

Up-regulation is a process taking place inside a cell, which is triggered by different signals results in increased expression of genes and as a result the proteins are encoded by those genes. On the other hand **down-regulation** is a process resulting in decreased gene and corresponding protein expression.

Up-regulation helps in re-establishing homeostasis. When a cell is deficient in some kind of receptor the process of up-regulation occurs. In this mechanism, more receptor protein is synthesized and transported to the membrane of the cell.When a cell is over stimulated by a neurotransmitter, hormone or drug for a prolonged period of time down-regulation occurs and to protect the cell the expression of the receptor protein is decreased.

Regulation of transcription thus controls how much RNA is to be created. Transcription of a gene by RNA polymerase can be regulated by mechanisms such as specificity of RNA polymerase for a given promoter altered by some specificity factors or set of promoters thus making it more or less likely to bind them (i.e. in prokaryotic transcription sigma factor is used).Repressor genes binds to the location where coding sequences on the DNA strand close to the promoter region impeding RNA polymerase's progress along the strand, thus impeding the expression of the gene.RNA polymerase is found to be located at the start of protein coding sequence by general transcription factors followed by the release of polymerase to transcribe the m-RNA.

Due to increase in interaction between RNA polymerase and a particular promoter region on DNA strand, increasing the attraction of RNA polymerase for a particular promoter region through interactions with subunits of the RNA polymerase.On the DNA helix there is one specific site called enhancer which are bound by activators in order to bring a specific promoter to the initiation complex. Enhancers are more common in eukaryote than prokaryotes. [39].

The function of mi-RNAs in cancer pathogenesis could be studied by Up or down-regulated expression of miRNAs. Due to fluctuation of a specific miRNA there is need to study role of the miRNA in cancer initiation and development. There are more than a few methods to carry out this study such as specific promoters, point mutation, antisense inhibitors and transgenics. Using antisense inhibitors to block the targeted miRNA function is a good example. In this strategy, an artificial antisense RNA competes with cellular mRNAs to bind miRNAs. The antisense RNA pairs with the miRNA and inhibits the miRNA function. This has been adopted by two different independent research groups to sequence-specifically inhibit miRNA and siRNA induced RNA silencing [40, 41] and inhibit four miRNAs in vivo by modified antisense RNAs [42]. Point mutation of miRNAs can be employed to study the function of miRNAs in cancers. One advantage of point mutation is to study the direct interaction of miRNAs and their targeted genes. One of the another sequence i.e. seed sequence is important for miRNAs to recognize their targets and the gene regulation function of miRNAs decreased by increasing the mismatch in the seed sequences [43, 44]. The nucleotide changes in the seed region of a specific candidate miRNA will notably decrease the possibility of the miRNA binding to its targets due to this mismatching it results in the over expression of targets. If these types of miRNAs or their targets are involved in cancer formation, this point mutation will affect the formation of cancer.

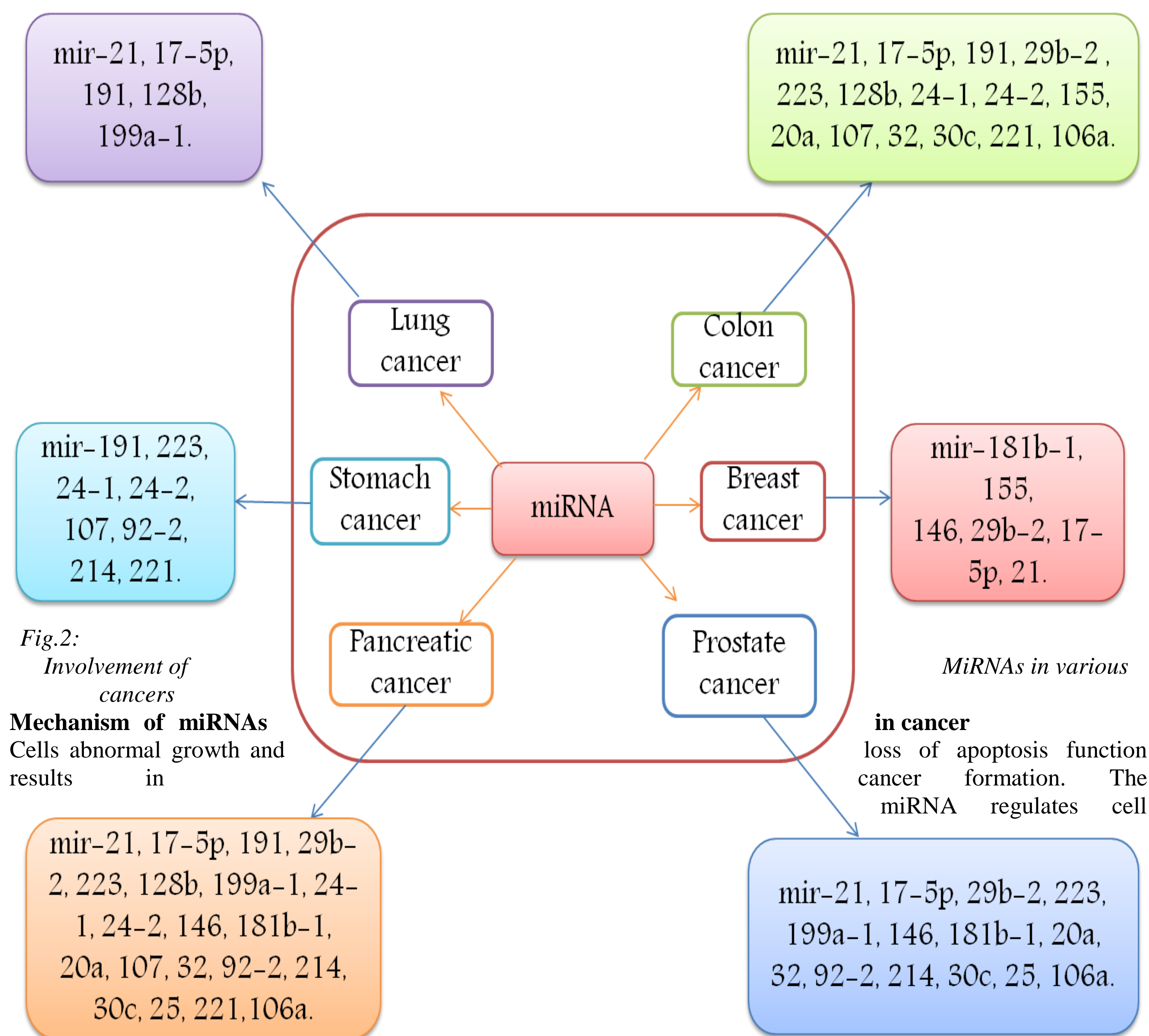

Fig.2: Involvement of MiRNAs in various cancers

Mechanism of miRNAs in cancer

Cells abnormal growth and loss of apoptosis function results in cancer formation. The miRNA regulates cell growth and apoptosis. i.e. by targeting antiapoptotic gene B-cell lymphoma 2 (BCL2) mRNA miR-15 and miR-16 induce apoptosis [45,46,47] which is a key player in many types of human cancers, including leukemias, lymphomas, and carcinomas [48]. Nairz et al. (2006) demonstrated that miss expression of miR-278 in developing eyes causes massive over growth in Drosophila due to inhibition of apoptosis by miR-278 [49]. By regulation of cell growth and apoptosis miRNAs are involved in some cancers. There are some evidence found against involment of miRNAs in cancers by studing molecular characterizing of 13q14 deletion in human chronic lymphocytic leukemia (CLL) [50]. It is the most common form of adult leukemia in the Western world. Two types of miRNAs i.e. miR-15a and miR-16a which are located on chromosome 13q14, a region deleted in more than half of B cell chronic lymphocytic leukemia (B-CLL) cases. These two miRNAs are the only two genes within the small (30 kb) common region which are lost in chronic lymphocytic leukemia patients and expression analysis indicated that miR-15 and miR-16a were either absent or down-regulated in the majority of CLL patients [51]. Recognition of miRNAs that are differentially expressed between tumor tissues and normal tissues helps to detect those miRNAs that are involved in human cancers and further stabiles the apparent pathogenic role of miRNAs in cancers [52]. Calin et al. (2004a,b) determined genome wise expression profiles of miRNAs in human B cell CLL using a micro array containing 368 probes corresponding to 245 human and mouse miRNA genes.

Micro array analysis of miRNA confirmed that miR-15 and 16 are reduced in human CLL [53-54]. In

further study micro RNA analysis also indicated that miRNA expression patterns were related to the clinical and biological behavior of CLL [55]. A recent study indicated that BCL2 is target of miR-15a and miR-16-1. Expression of miR-15a and miR-16-1 was inversely correlated to BCL2 expression in chronic lymphocytic leukemia; both miRNAs negatively regulate BCL2 at the post transcription allele. This was also confirmed in a leukemic cell line model [47] suggesting that miR-15a and miR-16-1 can be used therapeutically to cure tumors over expressing BCL2.

Responsible miRNAs in various cancers:

Lung Cancer:

Chemo-resistance, progression and Carcinogenesis of lung cancer are complex multi-step process resulting from deregulated gene expression following to accumulation of epigenetic or genetic abnormalities. By inactivating tumor suppressor genes or activating oncogenes these defects contribute to the malignant phenotype. MiRNA may be an important factor in those defects. The exact molecular mechanisms behind the altered expression of miR-21 in lung cancer are uncertain. Commonly for the prediction of miRNA target genes computational methods are used. It has been shown that the union of miRNA target genes predicted by three computational algorithms i.e. Target Scan, miRanda, PicTar is one of the strategies that give the highest sensitivity [56].

Acute myeloid leukemia:

Acute myeloid leukemia (AML) is a disease, characterized by proliferation and maturation arrest of myeloid blasts in bone marrow and blood [57]. The long-term overall survival rate for AML patients under the age of 60 years or older is 30–40% and under 10% respectively which remains a challenge [58]. Thus, it is needed to find out new targets for molecularly designed therapies. The patient with AML the expression of miR-370 was found to be down regulated in both leukemia cell lines and primary leukemic cells. Ectopic expression of miR-370 in HL60 and K562 cells led to cell growth arrest and senescence. In contrast, depletion of miR-370 expression using RNA interference enhanced the proliferation of those leukemic cells. Mechanically, miR-370 targets the transcription factor FoxM1, a well established oncogenic factor promoting cell cycle progression. Moreover, when HL60 and K562 cells were treated with 5-aza-20-deoxycytidine, DNA methylation inhibitor, miR-370 expression was up-regulated, which indicates epigenetic silencing of miR-370 inleukemic cells. By targeting FoxM1 the miR-370 function as a tumor suppressor and the epigenetic silence of miR-370 thus responsible for depression of FoxM1 expression and consequently due to that there is development and progression of AML [59].

Papillary thyroid carcinoma:

Papillary thyroid carcinoma (PTC) is the most generally observed malignancy in thyroid tissue, accounting for 80% of all thyroid cancers. Now a day the incidence of PTC patients have been increased in United States. [60]. In PTC there is alterations in the RETPTC-RAS-BRAF signaling pathway [61, 62]. In PTC tumors there is activating mutations in BRAF and RETPTC gene rearrangements occurs [63, 64]. Most of the case control studies of inherited genetic predisposition is showing that a 3 to 8 fold risk in first degree relatives, one of the highest of all cancers [65, 66].

The large families displaying Mendelian inheritance of PTC are rare mutations found even though several putative loci have been identified by linkage analysis [67–70]. Apart from alterations in the RETPTC-RAS-BRAF pathway, comparatively little is known about the genetics of PTC. It shows that numerous micro RNAs (miRNAs) are transcriptionally up-regulated in PTC tumors compared with unaffected thyroid tissue. A set of five miRNAs including the three most up regulated ones i.e. miR-221, 222 and 146 distinguished clearly between PTC and normal thyroid.

Additionally, studies found out that miR-221 were up-regulated in unaffected thyroid tissue in several PTC patients, presumably an early event in carcinogenesis. Tumors in which the up-regulation of miR-221, 222, and 146 was strongest showed dramatic deficit of KIT transcript and Kitprotein. In PTC cases, this down expression was associated with germ line single-nucleotide changes in the two recognition sequences in KIT for these miRNAs. The up-regulation of several miRs and regulation of KIT are responsible in PTC pathogenesis and that sequence changes in genes targeted by miRNA scan contribute to their regulation [71].

Colorectal cancer:

Colorectal cancer is a most common cancer worldwide that causes 655 000 deaths per year. In 2011, Colorectal cancer was accounts for 141 000 new cases and 49 000 deaths in the United States, making it the second leading cause of death from cancer.[72] Although early detection methods and therapeutic approaches have been studied for patient survival. The surgical removal of colonic part is the most successful treatment. After resection of colonic cancer part and if remain some microscopic stress of

neoplastic could develop cancer through metastatic [73] Colorectal cancer therapy resist cancer cell abundance can negatively affect adjuvant therapies, such as radiotherapy and chemotherapy.[74]

CONCLUSION:

Numerous studies demonstrated that the upregulation or down regulation of mi-rna leads to cancer. In addition, few mi-RNA such as 15a and miR-16-1 can be used therapeutically to cure tumors over expressing BCL2. However, the involvement of mi-RNA in various cancers needs to know the molecular mechanism of transcription and way of expression as much as possible to indulge the neoplasm. Thus, further studies will warranted to elucidate the potential culprits involved in cancers and other chronic diseases.

Conflict of interest:

The authors have no conflict of interests.

List of abbreviation:

13q14- Region of chromosome
30 UTR-30 Untranslated region
AML-Acute myeloid leukemia
B-CLL-B-cell chronic lymphocytic leukemia
Bcl-2-(B-cell lymphoma 2)
CHUK-Conserved Helix-Loop-Helix Ubiquitous Kinase
CLL-chronic lymphocytic leukemia
CRC-Colorectal cancer
DNA-Deoxy ribo nucleic acid
IKB- I-kappa-B-alpha
mi-RNA- Micro Ribo Nucleic acid
NF-κB-Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
PTC- Papillary thyroid carcinoma
RET- Rearranged during tansfection (RET)
RISC-RNA-Induced Silencing Complex
RNA- Ribo nucleic acid
si-RNA- Small interfering RNA (siRNA)

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