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IDENTIFICATION AND CHARACTERIZATION OF EVOLUTIONARY CONSERVED miRNAs IN VARIOUS TYPES OF CANCER: *IN SILICO* AND EXPERIMENTAL APPROACH

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ABSTRACT: The complex history of consensus sequence in microRNA family appears to be closely linked to the early evolution of primates. Each miRNA is predicted to target several hundred genes and the expression pattern of conserved miRNA among species through evolutionary time is an important phenomenon in cancer. Since miRNAs are major post-transcriptional negative regulators of target gene transcript; their origins and functional evolution in primates still remains unclear and controversial. Sixteen miRNAs from seven different types of cancers were analyzed to identify the conserved miRNAs in human and to assess their expression patterns. Evolutionarily conserved sequences were identified among primates for 16 miRNAs by ClustalV algorithm. The expression patterns of 16 miRNAs were confirmed by RT-qPCR. The consensus sequence match was observed in cancer with GU-rich region among globally expressed miRNAs. However, there was an AU-rich region was observed in all sixteen matured miRNAs. Moreover, GU-rich region was observed in stem-loop structured miRNAs. This result indicated that CU-region does not or less evolutionary conserved in cancer and it was replaced by other regions during evolution. The result reveals that these 16 miRNAs showed similar sequence conservation among primates and differentially expressed in seven different types of human cancer cell lines. The present research work reports the sequence conservation of globally expressed miRNA through evolution among primates located on the top branch of the phylogenetic tree and the conserved miRNAs are the major regulators in cancer.

KEYWORDS: Human evolution. Conserved miRNA. Cancer. ClustalV. Primates

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1. INTRODUCTION

MicroRNAs are small non-coding endogenous RNA evolutionarily conserved and consist of 18-22 nucleotides [1, 2, 3]. Functionally these are regulatory molecules and they negatively regulate the target mRNA post-transcriptionally by binding partial complementarity in 3'UTR leading to mRNA degradation and/or target translation inhibition. This negative regulator present in various species including humans, plants, animals, algae, fungi and some DNA viruses. The miRNA Let-7 of lin-14 mRNA is the first most identified small RNA involved in the development of C. elegance [4]. MicroRNAs are involved both in physiological and pathological conditions such as apoptosis, cell cycle, differentiation, metabolism, cancer and neurodegenerative disorders. However, the biological role and functions of most miRNAs are still poorly understood. In humans, miRNAs have been found to play an important role in embryogenesis [5], hematopoietic cell differentiation [6], and brain development. Thus, miRNAs have been implicated in the regulation of all crucial signaling pathways within a cell, and their aberrant expression has been shown to play an essential regulatory role in the pathogenesis of cancer [7]. The potential function of miRNA is still enigmatic, although functional miRNAs are identified as regulatory molecules and play an important role in various biological processes including cancer. The miRNA biogenesis starts in the nucleus and transcribed by RNA polymerase II, which forms the primary miRNA contains several kilobases. These primary miRNAs processed into 70-80 nucleotide containing hairpin-like stem-loop precursor transcripts by Drosha accompanied with DGCR8 (Digeorge syndrome critical region 8) [8]. After the completion of a primary process, the precursor miRNAs are transported into the cytoplasm via nuclear pore with the help of exportin [9]. Dicer that catalyses the cleavage of stem-loop of the precursor and forms passive miRNAs (*strand) and matured active miRNA. Then, the matured miRNA associated with the RNA Induced Silencing Complex (RISC) protein and repress or degrade the target mRNA [10]. The non-coding small RNAs are highly conserved once integrated into a gene regulatory network and are only rarely lost in the genome due to mutation [11, 12, 13, 14, 15]. The expansion of miRNA families in both basal lineage of bilaterian animal and lineage leading to placental mammals over evolution suggests that miRNAs are closely associated with organismal diversity [16]. In addition to the role of miRNA in ontogeny, some closely related evidence suggests that miRNAs have huge impacts on animal phylogeny. Studies on miRNA phylogenetic conservation and its functional diversity suggest that miRNAs play important roles in evolution, by driving phenotypic variation during development [17]. Primate miRNAs have a complex evolutionary history characterized by the deep conservation of some miRNA and frequent gain or loss of miRNA during evolution [18, 19]. Iwama et al. [20] reported that about 53% of the present human miRNA have originated within the simian lineage to human and approximately 28% were originated within hominoid lineage and only 15% were conserved beyond placental mammals. The species that covers the major lineage of primates and combined with the available data in human; chimpanzee (Pan Troglodytes), gorilla

(Gorilla gorilla) and orangutan (Pongo pygmaeus) has been selected for the present work to examine the evolutionary pattern of conserved miRNA sequences. miRNAs are broadly studied because of their crucial regulatory roles in biological processes and evolutionary players, especially for the conserved small miRNAs. Although they are characterized as the negative regulator, they are thought to have different evolutionary patterns. Homo sapiens miRNAs are conserved during evolution and it remains unclear. Hence, the present work is focused on comparing the sequence of human miRNA homologues with primates to analyze the nucleotides sequence similarities that shaped the evolutionary history of miRNA and its expression patterns.

2. MATERIALS AND METHODS

2.1. Cell Lines and Cell Culture

MCF-7, NCI-H23, HCT-116, MOLT-4, A498, SKMEL-28 and PC-3 cell lines were purchased from National Centre for Cell Science, India. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplement with 10 % Fetal Bovine Serum and 1 % penicillin-streptomycin antibiotics at 37 °C in a humidified atmosphere 95 % air and 5 % CO_2.

2.2. Identification of conserved sequence by ClustalV method using MegAlign 13 software

The sequence conservation and multiple sequence alignment among non-coding RNA transcripts were analyzed by the clustalV algorithm using MegAlign 13 software. The Homo sapiens miRNAs were compared with primates such as chimp, gorilla and orangutan. Primates were selected from the top branch of the phylogenetic tree to identify the conserved miRNAs through evolution. In brief, the 16 Homo sapiens miRNAs were selected from seven cancer cell lines. The selected miRNAs were subjected to identify the conserved sequence within the organ and were compared with primates. Ultimately, each miRNA were compared to identify sequence similarity with respect to cancer.

2.3. cDNA synthesis and RT-qPCR

Total RNAs were isolated from seven cancer cell lines using Trizol reagent (Invitrogen). The isolated total RNA (2µg) was used to construct cDNA using iScript cDNA Synthesis Kit. Real-time PCR assay was carried out using SYBR Green Master mix (Kappa, Hercules, CA) with specific miRNA stem-loop primers and universal reverse primer. Thermal cycler condition was used as follows: 40 cycles at 95 °C for 15 s, 60 °C for 45 s, and 72 °C for 15 s. All the matured and stem loop primers were designed according to Chen et al. 2005 [21]. The miRNA primers and its sequences used in RT-PCR are given in (Table 1). Expression levels of each matured miRNA were measured using the comparative threshold cycle (Ct) method as normalized to U6. The relative expression of each miRNA was calculated from the expression levels within the cancerous cell lines. Expression profiles of 16 miRNAs were analyzed by RT-PCR (Step One plus, Applied Biosystem). All 16 miRNAs were selected for the analysis based on their deregulatory roles in association with promotion and progression of cancer.

S.No	miRNA	Sequence
1.	miR-195	Forward Primer : GCGCGGTAGCAGCACAGAAATA Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGTGCCAA
2.	miR-497	Forward Primer : GCGCGGCAGCAGCACACTGTG Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : TCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGACAAAC
3.	miR-215	Forward Primer : GCGCGGATGACCTATGAATTG Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGGTCTGT
4.	miR-192	Forward Primer : GCGCGGCTGACCTATGAATTG Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGGGCTGT
5.	miR-17*	Forward Primer : GCGCGGACTGCAGTGAAGGCAC Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGCTACAA
6.	miR-18a*	Forward Primer : GCGCGGACTGCCCTAAGTGCTCC Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : TCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGCCAGAA
7.	miR-508-5p	Forward Primer : GCGCGGTACTCCAGAGGGCGTCA Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGCATGAG
8.	miR-509-3-5p	Forward Primer : GCGCGGTACTGCAGACGTGGCA Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGCATGAT
9.	miR-509-5p	Forward Primer : GCGCGGTACTGCAGACAGTGG Reverse Primer : AACTGGTGTCGTGGAG

		Stem Loop : TCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGTGATTG
10.	miR-510	Forward Primer : GCGCGGTACTCAGGAGGAGAGTGG Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGTGATTG
11.	miR-193a-3p	Forward Primer : GCGCGGAACTGGCCTACAAAGT Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGACTGGG
12.	miR-452	Forward Primer : GCGCGGAA1CTGTTTGCAGAGGA Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGTCAGTT
13.	miR-30e	Forward Primer : GCGCGGTGTAAACATCCTTGAC Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGCTTCCA
14.	miR-30c	Forward Primer : GCGCGGTGTAAACATCCTACACT Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGGCTGAG
15.	miR-196b	Forward Primer : GCGCGGCTGGGAGAAGGCTGTT Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGAGAGTA
16.	miR-30c-2*	Forward Primer : GCGCGGCTGGGAGAAGGCTGTT Reverse Primer : AACTGGTGTCGTGGAG Stem Loop : CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGAGAGTA

Table 1. miRNA Primer sequences used in Quantitative Real Time-PCR

2.4. Statistical analysis

Statistical significant were analyzed for three independent RT-qPCR experiments (n = 3) by One-way ANOVA using GraphPad Prism software version 6.0.

3. RESULTS AND DISCUSSION

3.1. Multiple Sequence Alignments for sixteen miRNAs

Sixteen miRNAs from seven different cancer cell lines were analyzed for their sequence conservation and their expressions pattern. To analyze the sequences conservation, miRbase was used for the miRNA sequence identification (www.mirbase.org). MiRNA sequence alignment was performed by MegAlign 13 software using Clustal V algoritham to identify the consensus sequence in different types of cancer. In breast cancer, miR-195 and miR-497 showed the conservation in 5' arm. In colon cancer cell line, both miR-192 and miR-215 had similar sequence arrangements and are highly conserved. However, miR-17* and miR-18a* were less conserved in leukemia MOLT-4 cell line. In melanoma, miR-508-5p, miR-509-3-5p, miR-509-5p and miR-510 showed high sequence conservation. Moreover, miR-193a-3p and miR-452 had highly conserved sequence. Both miR-30e and miR-30c expressed in prostate cancer showed high sequence conservation at 5' end. In A498 cells, miR-196b and miR-30c-3* had the less conservation in their sequence. The consensus sequence match was observed in cancer with GU rich region among miRNAs (Fig. 1). However, consensus match was observed in AU and GC rich region among all sixteen matured miRNAs (Fig. 2).

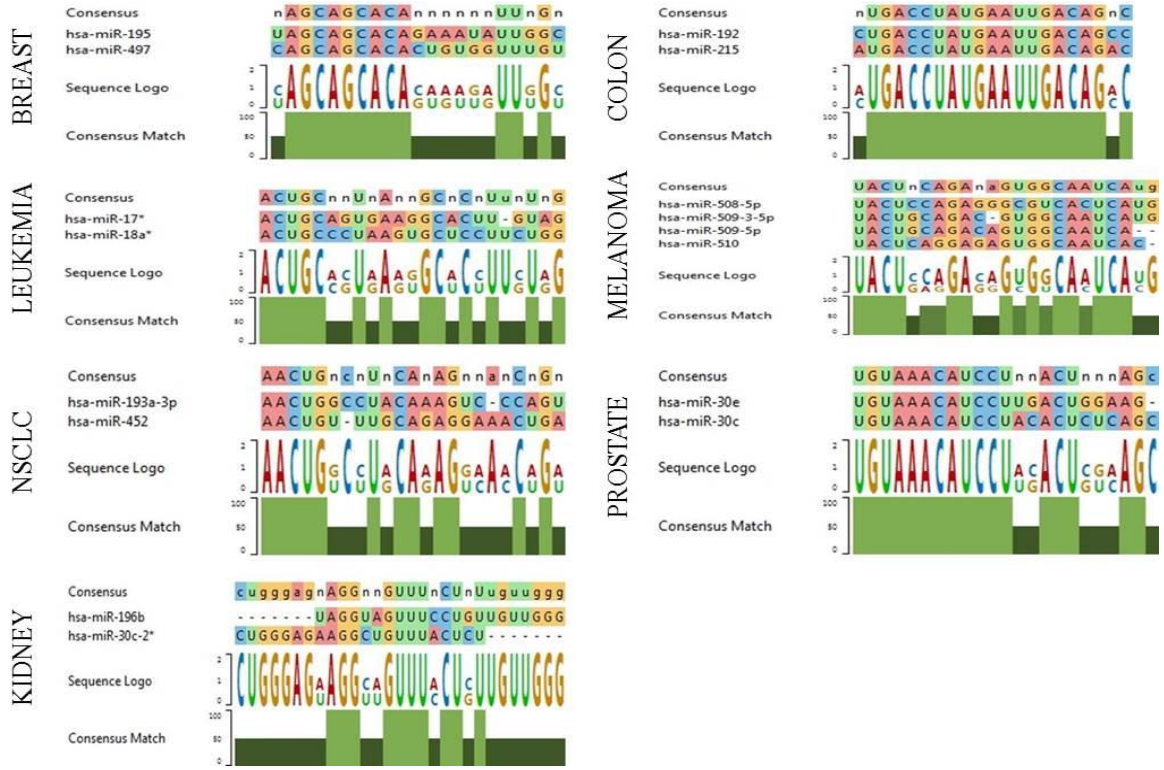


Figure 1. The consensus miRNA sequence in different types of human cancer. The consensus sequence match was observed in various types of cancer with GU-rich region among miRNAs. The consensus match bar (green) shows high sequence conservation. Nucleotides of miRNAs that do not match their genomic sequence are mentioned as "n".

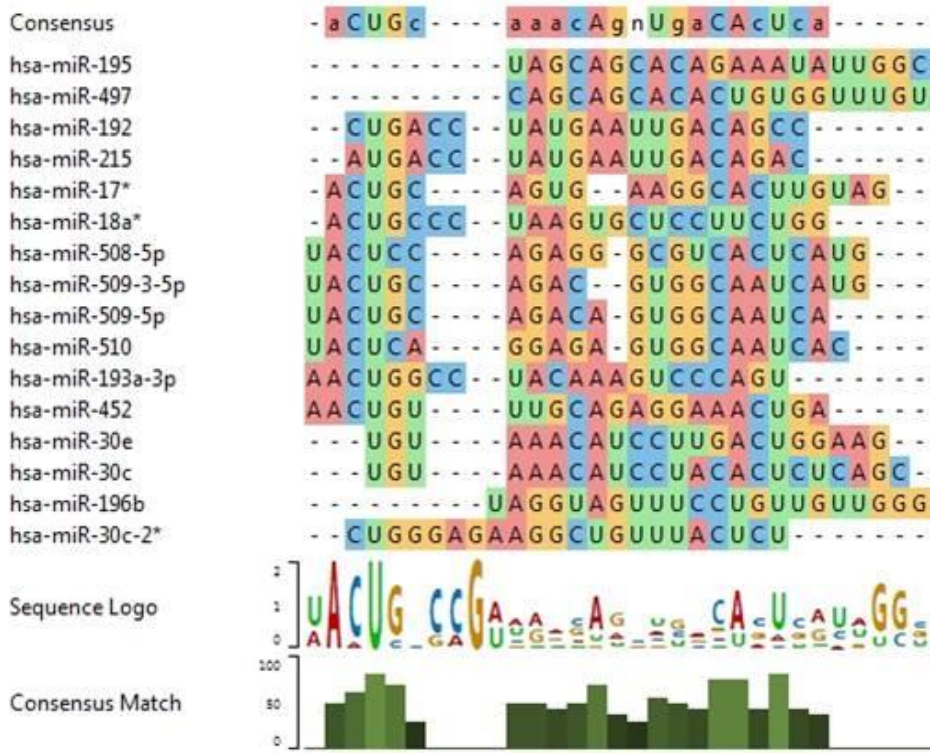


Figure 2. Multiple sequence alignment of cancer associated human matured miRNAs. We selected 16 miRNAs from various types of cancer cell lines. The AU and GC rich region was observed in all sixteen matured miRNAs. The consensus match bar (green) underneath sequence logo shows the degree of sequence conservation.

Moreover, GU-rich region was observed in stem-loop structured miRNAs (Fig. 3).

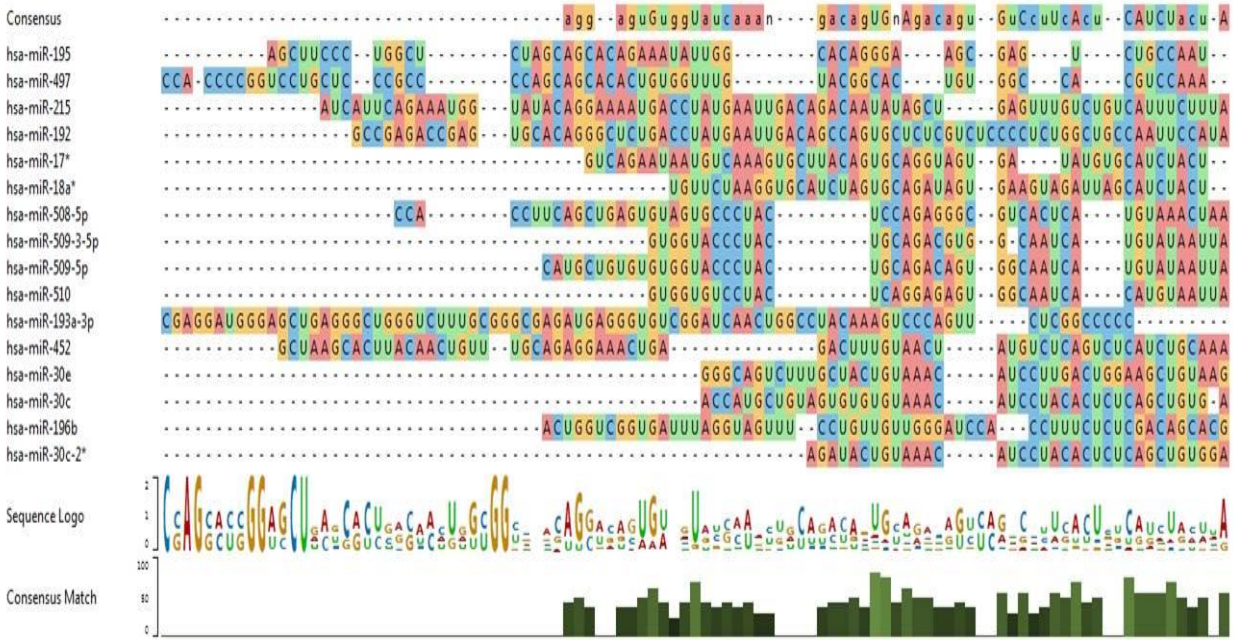
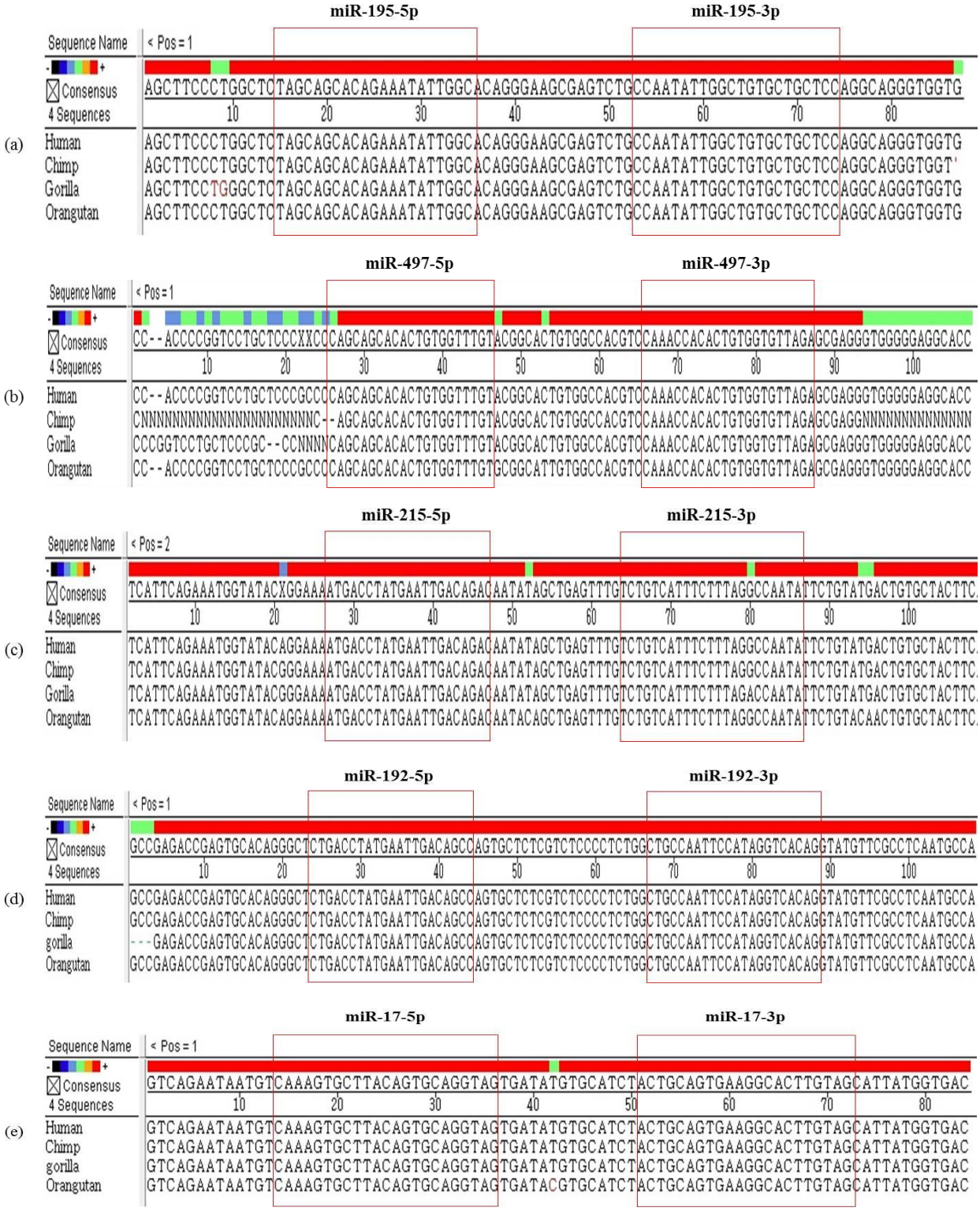


Figure 3. Multiple sequence alignment of cancer associated human stem-loop miRNAs. Conserved positions shown in the sequence alignment. Consensus ruler (green) shows the conserved sequence.GU-rich region was observed in stem-loop structured miRNAs. The consensus match bar

(green) underneath sequence logo shows the degree of sequence conservation.

This result indicated that CU-region does not or less evolutionary conserved and it was replaced by other regions during evolution. In order to identify the conserved miRNA over the human evolution, each human miRNA sequences were compared with three primates located on the top branch of phylogenetic tree. All 16 human microRNAs has the evolutionarily close relationships with Chimp, Gorilla and Orangutan. Human miRNA sequences have the sequence similarity with primates both in active and passive strands (Fig. 4).



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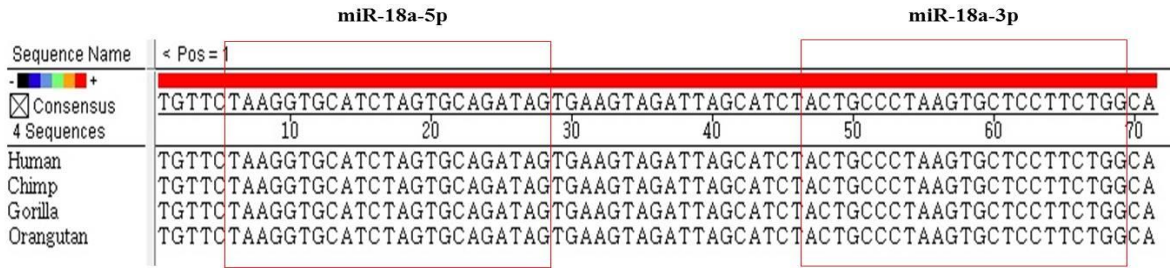

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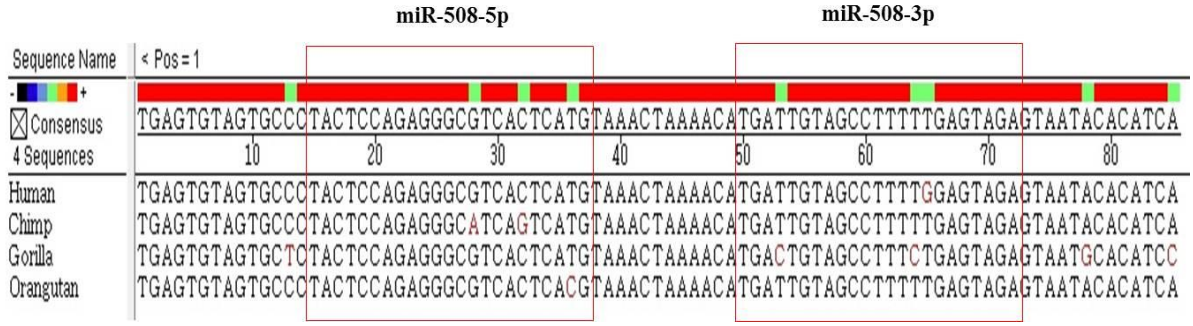

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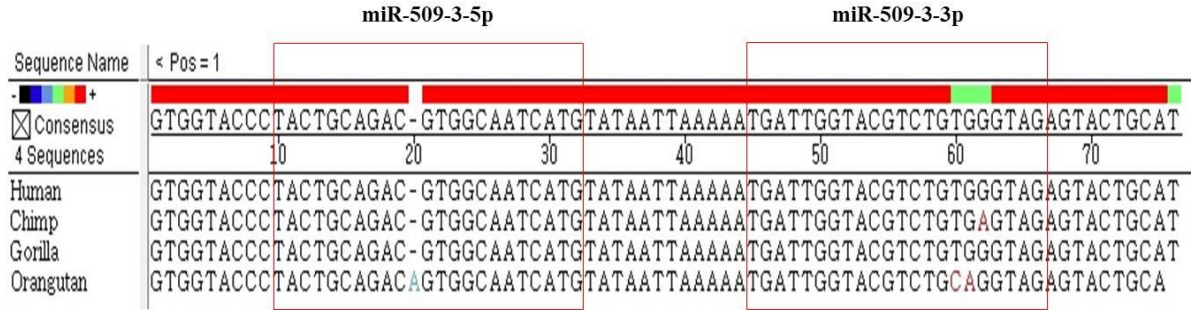

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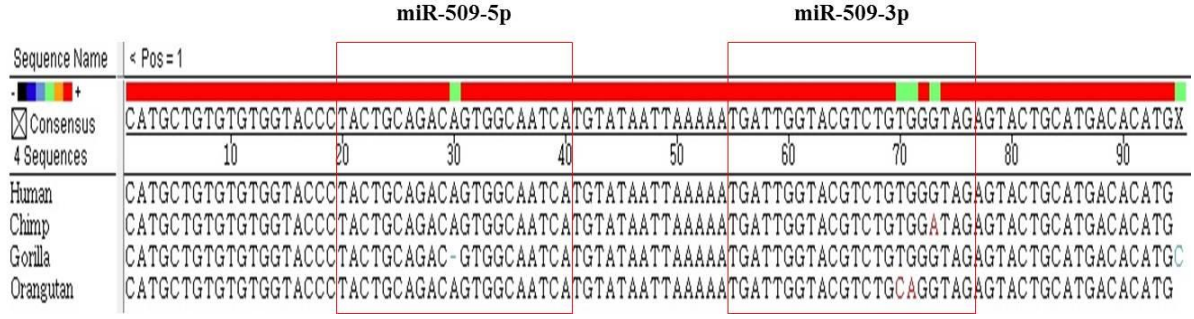

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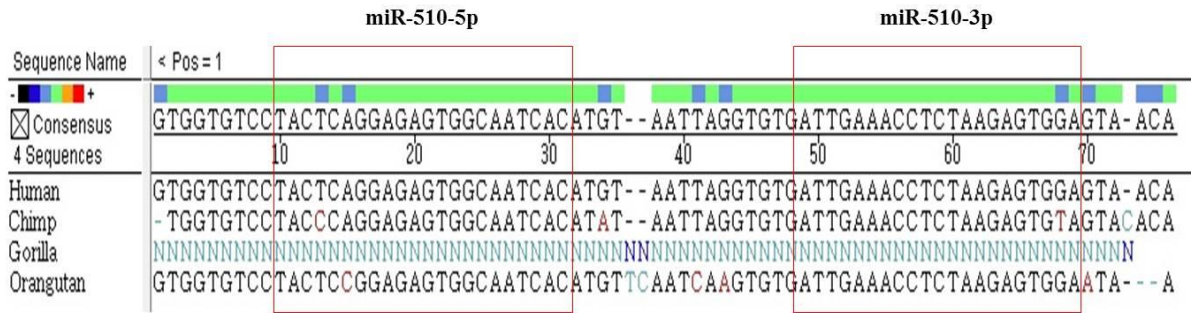

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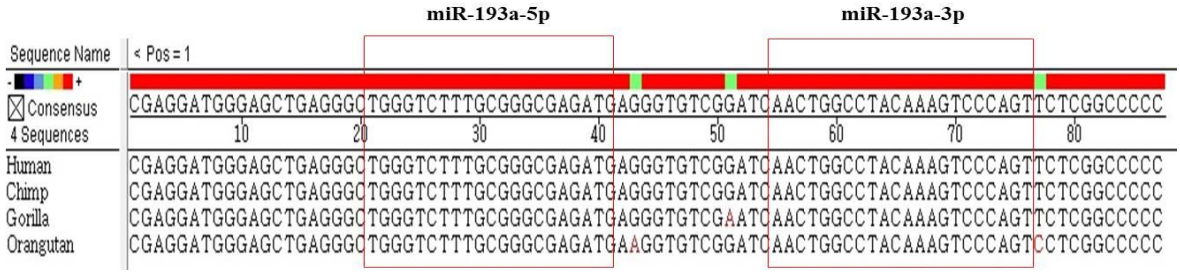

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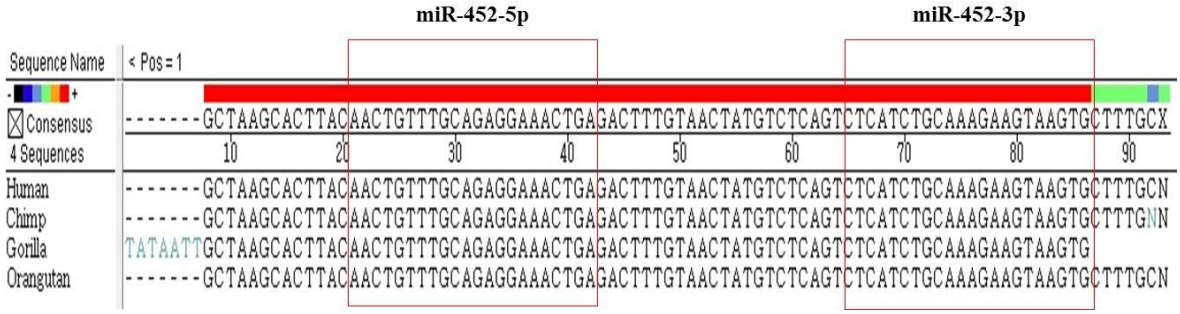

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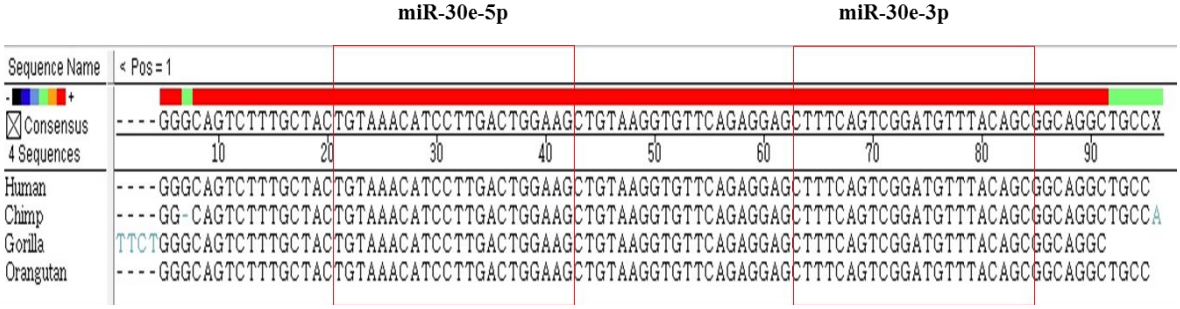

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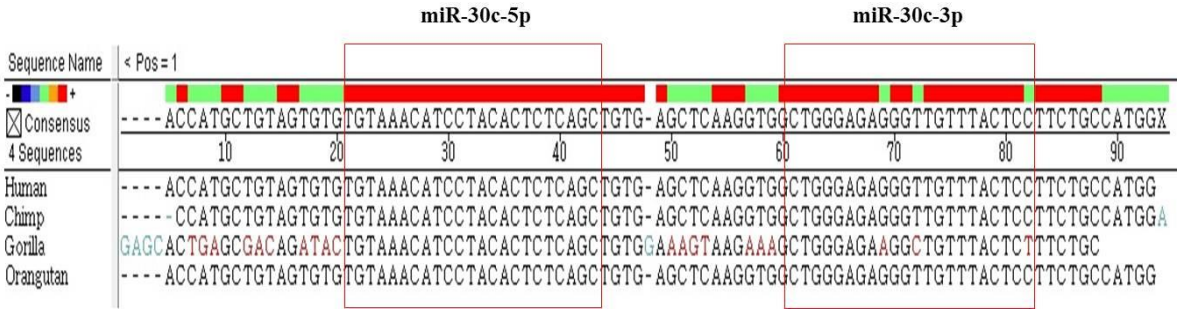

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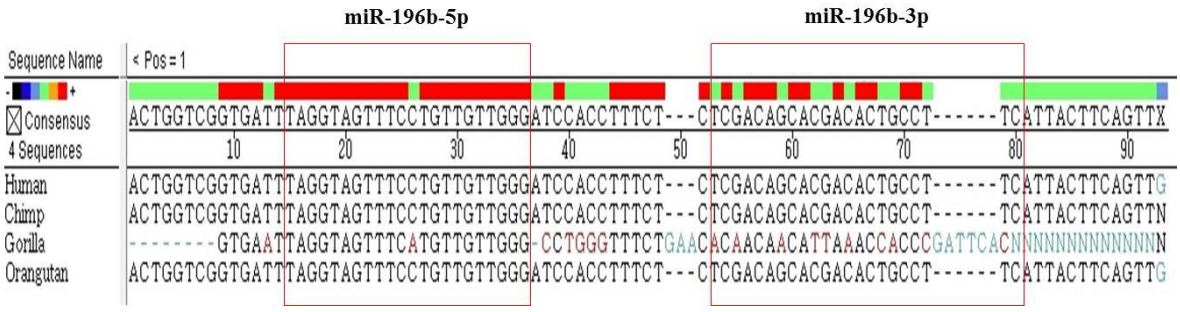

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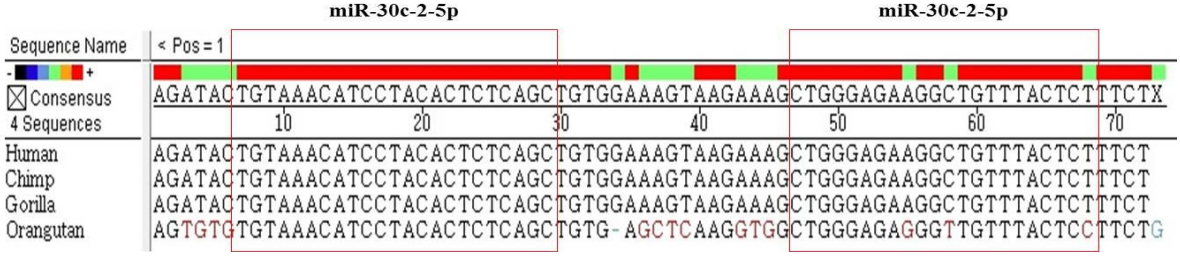

Figure 4. miRNAs were conserved over the human evolution. All 16 human microRNAs has the evolutionarily close relationships with Chimp, Gorilla and Orangutan. Each human miRNA sequences were compared with 3 primates. a, b. Breast; c, d. Colon; e, f. Leukemia; g, h, i, j. Melanoma; k, l. Lung; m, n. Prostate; o, p. Kidney. Sequences of primates were obtained from www.mirbase.org. Multiple sequence alignment was performed using ClustalV method using Megalign software. Human miRNA sequences have the sequence similarity in both active and passive strand in all species. Quotation mark (') means that there is no sequence present in the species. Sequences (red) are evolutionarily altered in the species. Mark (N) means that there is no sequence present or deleted in the species and sequences showed in light blue are evolutionarily added.

3.2. Functional diversification of miRNA in cancer

miRNAs have been implicated in many critical biological processes. The aberrant expression of miRNAs observed in human cancers suggests that miRNAs may play an important role in tumorigenesis. Total RNA were isolated to examine the expression of sixteen miRNAs in seven different cancer cell lines to uncover the regulatory role of miRNAs. miRNAs expressions were analyzed by RT-qPCR. There were no similarities among miRNA expression pattern in different types of cancer. However, only miR-215 & miR-192 were expressed with same Cq value in colon cancer (Fig. 5). The similarity in expression is due to the presence of high sequence match between miR-215 & miR-192. In contrast to miR-215 & miR-192 expression, other miRNAs (miR-195, miR-497, miR-508-5p, miR-509-3-5p, miR-509-5p, miR-510, miR-193a-3p, miR-452, miR-30e, miR-17*, miR-30c and miR-18a*) have showed invariably higher Cq expression values (Fig. 5).

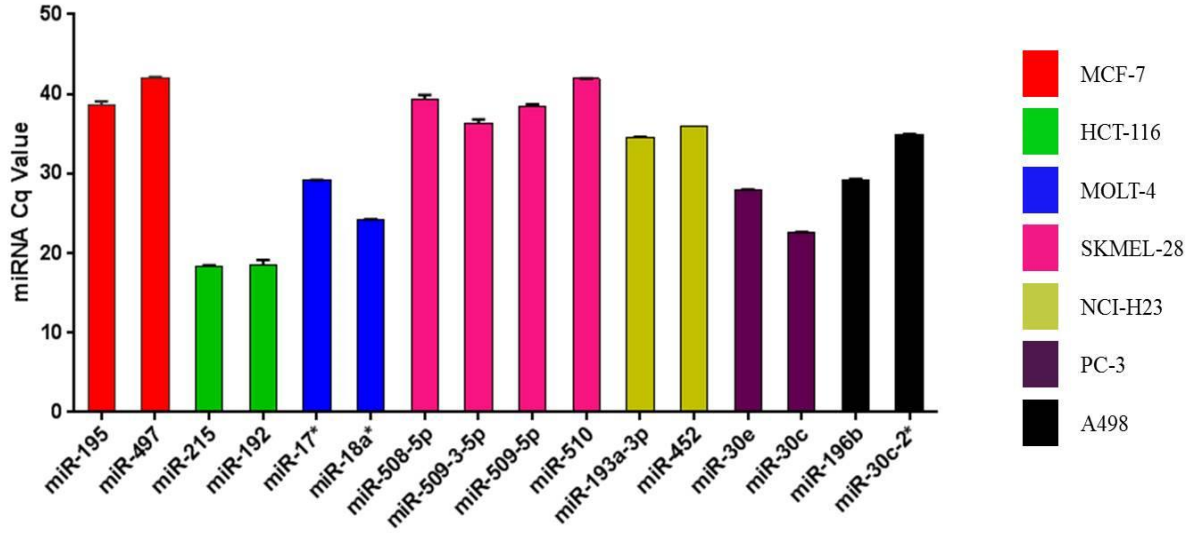

Figure 5. miRNA expression in different types of cancer cell lines

Differential expression of 16 miRNAs were evaluated to analyses the comparative threshold cycle (Ct) by RT-qPCR. The relative expression of each miRNA was calculated from the expression levels within the seven different types of cancerous cell lines.

In the present work, we made a systematic comparison of conserved and non-conserved sequence

alignment for 16 miRNAs from human, chimp, gorilla and orangutan located on the top branch of the phylogenetic tree. Previous studies have shown that non-conserved pre-miRNA from human, mouse and chicken had more GC than AU contented but conserved pre-miRNA was rich in AU [22]. In the present study, the results indicated that both active and passive strand of miRNAs provides the conserved sequence similarity among primates compared to humans. Besides, the higher degree of AU and GC content of conserved sequences of miRNAs from three primates with human was predominantly notable and suggesting that these feature might be through miRNA evolution.

The present study revealed the relative expression of sixteen miRNAs including three passive strand miRNAs such as miR-17* and miR-18a* in MOLT-4 and miR-30c-2* in A498. The end process of the miRNA passive strand in mature miRNA biogenesis, either expressed abundantly as a potential functional guide miRNA or degraded to a passive strand, may be destined across evolution [23]. Besides, the well-conserved miRNA* strands in seed sequences may afford potential opportunities for contributing to the regulatory network [24]. Both miR-17* and miR-18a* showed a less sequence conservation even they expressed in the same cell lines. The miR-30c-2* expressed in A498 has less conserved sequence. The differences in expression of mature miRNAs were evaluated for sixteen miRNAs that are specifically expressed in seven various types of cancer cell lines. All the 16 miRNAs were differentially expressed among the cancer cell lines. Some miRNAs showed the similar levels of expression (Fig. 5). Even all the cancer cell lines exhibited diversification in its expression, miR-508-5p, miR-509-3-5p, miR-509-5p and miR-510 that were expressed in SKMEL-28 showed high expression level. In addition, both miR-215 and miR-192 with no differential expression was observed in HCT-116 cell line. All of these results relate a possible role of miRNAs in promoting cancer. Patnaik et al. [25] have identified that 72 miRNAs are highly deregulated in the NCI-60 panel of human cancer cell lines. Hence, the present study clearly suggests that miRNAs have different sequences and the selected sixteen microRNAs are conserved in different types of cancer. All sixteen Homo sapiens miRNAs were aligned to sequence homology compared to primates such as Chimp, Gorilla and Orangutan. Each Homo sapiens microRNA genes were analyzed with respect to alignments of primate genomes from the miRBase genome browser and found that all miRNAs showed conservation over the primate evolution. Based on the results of sequence comparisons, all 16 human microRNAs has the evolutionarily close relationships with Chimp, Gorilla and Orangutan. These finding demonstrated that all Homo sapiens miRNAs had the sequence similarity with primates (Fig. 4). The Primate and Homo sapience oncogenic miRNAs have retained their harmful regulatory interaction with target genes through their evolution because miRNA-mRNA interaction pave the way for the loss of newly formed miRNA and or causes the elimination of detrimental miRNA binding sites [16, 26, 27, 28]. The expression of miR-195 and miR-497 were highly conserved in miRNA cluster and located at chromosome 17p13.1 and were down-regulated by DNA methylation and increased CpG methylation in breast cancer [29]. Besides, miR-195 inhibits the cancer hallmarks

(proliferation, invasion and metastasis) by altering the lipogenesis in breast cancer cells [30]. MiR-497 suppressed the cancer cell growth, migration and invasion by arresting G1 phase of cell cycle through targeting cyclin E1 [31] and induce apoptosis by negatively regulating bcl-2 [32]. In our present study, miR-195 and miR-497 were highly expressed in breast cancer cell lines and proving the importance of these miRNA in breast cancer fate. The expression level of miR-215 was down-regulated in colon cancer and associated with patient survival by targeting thymidylate synthase (TS), dihydrofolate reductase (DHFR), and denticleless protein homolog (DTL). Khella et al. [33] reported the sequence comparison of miR-192 and miR-215 among species and these two miRNAs are highly conserved in 28 species. Both miR-192 and miR-215 induces cell cycle arrest and inhibits cell proliferation by targeting transcriptional thymidylate synthase (TYMS) in colon cancer cell line. This may be due to the similarity of seed sequence of both miRNAs that are important for the miRNA-mRNA target interaction [34]. Moreover, elevated levels of miR-215 subjected to chemo-resistance due to cell cycle arrest and reduced cell proliferation. Thus, miR-215 is a potential prognostic biomarker in colon cancer [35]. The miR-192 is frequently down-regulated in colorectal cancer and reduced expression leads to patients with the larger tumor in size. MicroRNAs showed differential expression in cancer cell lines due to their regulatory activities. Acunzo and Croce et al. [36] reported the involvement of tumor suppressor miR-15a and miR-16-1 in chronic lymphocytic leukemia (CLL) and are expressed in the same polycistronic RNA. MiR-17-3p down regulates pyruvate carboxylase and promotes cancer cell proliferation in gall bladder cancer cells [37]. Moreover, miR-17-3p targeting mitochondrial antioxidant proteins manganese superoxide dismutase (MnSOD), glutathione peroxidase-2 (GPX2) and thioredoxin reductase-2 (TrxR2) suppresses tumourigenesity [38]. Besides, miR-17-3p upregulates LDH and enhances glycolytic pathway in prostate cancer by inhibiting mitochondrial metabolism [39]. Mogilyansky et al. [40] revealed that miR-17-5p and miR-17-3p were up-regulated in myeloid leukemia and were down-regulated in acute myeloid leukemia. MiR-18a is one of the members of miR-17-92 cluster family and is highly conserved among vertebrates. MiR-17 and miR-18a seed families are not found outside of vertebrates [41]. MiR-18a acts as an oncogenic role in acute leukemia and was located on chromosome 13q31. Over expression of miR-18a enhanced the colony-forming capacity of mouse bone marrow progenitor cells and it targets Smad2 and Smad4 in TGF-β signaling pathways [40]. Previous studies have shown that miR-18a was over expressed [42] and also down regulation of miR-18a sensitizes lung cancer cells to radiation treatment [43]. They are highly elevated to promote cancer through directly targeting STK4 gene and suppress apoptosis process [44]. MiR-509-5p activates Mdm2/p53 pathway and promotes cancer of the cervix and hepatocellular carcinoma. Over expression of miR-509-5p suppresses the growth, cell migration and invasion of cervical cancer cells and it also regulates apoptosis and G1/S-phase transition of cell cycle [45]. The miR-509-5p expression was increased in stage iv melanoma than miR-508-5p [46]. Liu et al. [47] have identified a set of the miR-509-miR-514 cluster, including miR-

509-3p, compared to other cancer tissues, miR-509-3p expression was high in melanomas and located on chromosome Xq27.3 in the human genome, very close to the Melanoma Antigen family A genes and CSAG2, and these mRNAs are key regulators in melanoma. MiR-510 was highly elevated in breast cancer that lead to its tumourigenesity by increasing their cell proliferation, migration and invasion properties by the negative regulation of PRDX1 and positive regulation of PI3K/Akt signaling pathway [48]. Zhang et al. [49] observed rapid sequence evolution of the miRNAs in primates and miR-510 was highly expressed in melanoma. Moreover, miR-508-5p, miR- 509-3-5p, miR- 509-5p and miR-510 were highly conserved among primates. Down regulation of miR-193a-3p and miR-193a-5p associated with tumor and lymph node metastasis targeting through ERBB4/PIK3R3/mTOR-S6K2 signaling pathways [50]. MiR-193a has been reported as a tumor suppressor in cancer and it also act as an anti-oncogenic miRNA by downregulating the expression of WT1 that negatively regulates E-cadherin protein level and thus miR-193a prevents epithelial-to-mesenchymal transition by modulation of WT1-E-cadherin axis in non-small cell lung cancer [51]. Aberrant expression of miR-193a-3p regulates AEG-1that induces carcinogenesis in non-small cell lung cancer patients [52]. Down-regulated expression of miR-452 negatively correlates by regulating BMI1 gene with advanced tumor stage and lymph nodes metastases in non-small cell lung cancer [53]. In prostate cancer, miRNAs are aberrantly expressed and it also contributes to the progression, initiation and metastasis. Thus, miRNAs act as a biomarker for the early detection of cancers, including prostate [54]. MiR-30e negatively regulates BIRC6 and AVEN, which suppresses the apoptotic activity and it also inhibits Beclin-1 and suppress autophagic process in glioblastoma cell lines [55]. MiR-30c is the member of miR-30 family which inhibited the progression of colon cancer growth by regulating ADAM-19 gene expression [56]. MiR-30c targets several multifaceted cancer associated genes UB69, MTA1 and HBOX1 in prostate cancer. Ling et al. [57] have examined the miR-30c expression patterns in prostate cancer and found that the miRNA expression significantly decreased and it was also used as a prognostic tool to detect prostate cancer [58]. Cabarcas et al. [59] have identified that the highly expressed miR-30e targets sex-determining region Y box 4 (Sox4) and BCL9 in prostate cancer cell lines. NF-kB activation is one of the important features in prostate cancer. An activation of NF-kB regulates proliferation and survival of prostate cancer cells. MicroRNAs are main regulators of NF-kB pathway where miR-30e directly target and abrogates the functions of IkBα, a potential inhibitor of NF-kB in prostate cancer. Moreover, miR-30e and miR-30c are evolutionary conserved in varies vertebral species [59, 60, 56]. In ovarian cancer cells, miR-30c-2-3p targets Activating Transcription Factor 3 (ATF-3) and induces cell proliferation that leads to cancer progression [61]. MiR-30c-2* is located on chromosome 6q13 directly targets HIF2α. Mathew et al. [62] reported that miR-30c-2* function as tumor suppressive role in clear cell renal carcinoma. HIF2α regulates von-hippel Lindau (VHL), inactivation of the VHL gene occur in 90% of human clear cell renal carcinoma and leads to the stable expression of HIF2α. Several studies have shown that miR-

196b play a dual role as an oncogenic and/or tumor suppressor in several types of cancer. In oral squamous cell carcinoma patients, over expressed miR-196b contributes to cell migration and invasion due to aberrant DNA hypo-methylation of miR-196b in oral cancer [63]. Also MiR-196b is located within the HOX gene cluster and highly evolutionary conserved in human and mice. MiR-196b was overexpressed in renal cell carcinoma and it embedded within a CpG island, and overexpression in GC is associated with hypo-methylation in cancer [64]. Even though Berezikov et al. [65] have identified the sequence conservation for 122 miRNA, the globally expressed 16 human miRNA (except miRNA-195, miRNA-215, miRNA-509-5p and miRNA-30c-2) in cancer has not been reported. Based on the results described, our data suggests that sixteen microRNAs showed the functional diversification in their expression. These findings indicated that miRNAs have consensus nucleotide distribution among primates and expressed inconsistently across seven different cancer cell lines with functional divergence patterns.

4. CONCLUSION

The present study demonstrates that the globally expressed human miRNA is conserved among primates and are differentially expressed in cancer. Homo sapience and Primate specific conserved miRNAs may be a key sources to identify cancer therapeutic targets, particularly in cells and tissues of primate and human lineages which undergone prominent genomic modification. To the best of our knowledge, we are the first to report the sequence conservation in miRNA through evolution among primates located on the top branch of the phylogenetic tree and moreover the conserved miRNAs are the major regulators in cancer. In conclusion, the identification and characterization of conserved miRNA in cancer provides the important role of miRNA and are the therapeutic target for the treatment.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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