Chapter 5

Liquid Biopsies in Multiple Myeloma

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Abstract

Multiple myeloma is the second most common hematological malignancy. It is a heterogeneous disease characterized by focal lesions of malignant plasma cells in the bone marrow. Since bone marrow biopsy is a single-site procedure, its potential is limited in discovering the many clones present in each patient. Thus, a new approach, liquid biopsy, seems to be more relevant in today's world. Liquid biopsy can analyze circulating tumor cells or various circulating molecules (cell-free DNA, microRNA, long non-coding RNA and many others) that originated from the various tumor sites and thus will represent many different subclones. This review summarized current situation in research of liquid biopsies in multiple myeloma.

Keywords: multiple myeloma, liquid biopsy, non-coding RNA, circulating microRNA, cell-free DNA, circulating plasma cells

1. Introduction

Monoclonal gammopathies (MG) are a group of diseases characterized by proliferation of clonal plasma cells (PC). Physiological PC are terminally differentiated B cells, which secrete various types of antibodies used for neutralization of pathogens [1]. This essential function of PC is disrupted in MM patients, and abnormal cells overproduce monoclonal immunoglobulin (M-Ig) [2]. Multiple myeloma (MM) and its precursor disease monoclonal gammopathy of undetermined significance (MGUS) are two most common MG. The less frequent MG include Waldenström macroglobulinemia, solitary plasmacytoma, light chains amyloidosis and plasma cell leukemia [3].

MM is the second most common hematological malignancy [4]. It is caused by malignant transformation of PC, which infiltrate the bone marrow (BM) disrupting normal hematopoiesis. Moreover, they produce M-Ig that is found in serum and/or urine of MM patients. MM is characterized by a set of clinical features known as CRAB features (hypercalcemia, renal failure, anemia and bone lesions) [5, 6].

MM represents about 13% of all hematological and about 1% of all malignancies. The incidence in the Czech Republic has been reported at 4.8/100000 per year [7], while in Europe it is slightly higher, 6/100000 per year [8]. Interestingly, MM is quite common in North America, Europe and Australia, while it is rare in the Middle East and Asia [9].

In 2003, the International Myeloma Working Group (IMWG) published diagnostic criteria for MM: infiltration of BM by malignant PC >10%, CRAB features and presence of M-Ig in serum and/or urine [2]. Thus, MM was treated only when CRAB feature(s) were fully developed. At that time, most treatment options were quite toxic, which is why treatment was postponed [10]. The past 10 years, however, have brought unprecedented new treatment options (immunomodulatory drugs, proteasome inhibitors, monoclonal antibodies) that improved survival rates as well as quality of life of MM patients. This led to revision of diagnostic criteria in 2014 from diagnostics based on clinical symptoms to diagnostics based on biomarkers allowing earlier treatment of the disease [6].

Since the new drugs have been introduced into clinics, detection of minimal residual disease (MRD) has become even more important as MRD negativity is a prognostic factor in MM. Moreover, sensitivity of the detection method is becoming an issue since new drugs induce deep response, and MRD needs to be measured with sensitivity up to 10^{-6}. The two most common methods used for MRD detection are multiparametric flow cytometry and ASO-PCR (allele-specific PCR) [11–13]. ASO-PCR is based on the detection of patient-specific V(D)J rearrangement in bone marrow plasma cells (BMPC) [14]. Nowadays, next-generation sequencing (NGS) is gaining more attention as a more precise and modern method of detection. However, NGS needs to be standardized and more accessible before it can be used more broadly.

The genome of MM cells is highly unstable and harbors various cytogenetic abnormalities, including translocations, deletions or duplications. From the cytogenetic point of view, MM may be divided into two groups: hyperdiploid and non-hyperdiploid. Hyperdiploid genome is mostly characterized by trisomies of odd chromosomes (3,5,7,9,11,15,19,21) and is connected to better prognosis [5], while non-hyperdiploid genome is characterized by monosomies of chromosomes 8,13,14,16,17 and 22 and recurrent chromosomal translocations involving the immunoglobulin heavy chain (IgH) locus at 14q32. In MM, the most frequent chromosomal translocations are t(11;14)(q13;q32) (15–20% of MM patients) and t(4;14)(p16;q32) (12–15% of MM patients). Other translocations are less frequent, found only in less than 5% of patients (t(14;16)(q32; q23), t(14;20)(q32;q11) and t(6;14) (p21;q32)). In MM cells with t(11;14)(q13;q32), cyclin D1 gene is translocated under the control of immunoglobulin heavy chain enhancer. Similarly, cyclin D3 gene at 6p21 is overexpressed in MM cells carrying t(6;14)(p21;q32) [14].

In addition to MM being a genetically heterogeneous disease, it is also characterized by multifocal tumor deposits throughout the BM and focal lesions elsewhere. Malignant PC in these lesions carry various cytogenetic aberrations with varying level of prognostic value [15].

Diagnosis and monitoring of MM are routinely performed using BM aspiration and/or BM biopsy [6]. However, discrepant results were described from analyses of different biopsy sites within the same patient [16, 17]. Diagnostic biopsies of the BM are obtained only from a single site in the BM what creates a sampling bias and provides only a limited molecular profile as all subpopulations of PC, so-called subclones, are not present in the BM [18].

Liquid biopsies represent one of the possible solutions for more comprehensive analysis of MM patients. Various targets, which can be analyzed in MM samples, include circulating tumor cells [19], cell-free DNA (cfDNA) [20], microRNA (miRNA) [21] and long non-coding RNA (lncRNA) molecules [22]. This review summarizes current knowledge of all aspects of liquid biopsies in MM.

2. Circulating plasma cells

In some cases, PC may migrate out of the BM into peripheral blood (PB), then they are called circulating PC (cPC) [23, 24]. While the reason for the migration is unclear, it is clear that these cPC lose (in some cases only temporarily) their dependence on the BM microenvironment due to the loss of adhesion molecules, increased proliferation, increased number of chromosomal aberrations and increased angiogenesis [25, 26]. The presence of cPC in newly diagnosed MM patients has been associated with shorter survival of patients, and it is an independent negative prognostic factor [24]. It is also possible that it is the first feature of extramedullary relapse, which is characterized by infiltration of PC into soft tissues and bad prognosis for patients [27]. In case that the number of cPC increases to over 20% in PB, it may turn into progression to secondary plasma cell leukemia [28].

cPC can be detected in a small fraction of newly diagnosed MM patients (15%) by conventional morphology [29]. However, this frequency increases up to 50–70% once more sensitive techniques, such as flow cytometry, are used [30]. Interestingly, the presence of cPC has been associated with an increased risk of malignant transformation to symptomatic MM in MGUS patients as well as with an inferior survival among symptomatic newly diagnosed and relapse/refractory MM [31]. In a study by Paiva et al., cPC were analyzed by multiparametric flow cytometry, FISH and cell cycle analysis; cPC were compared to paired PC samples from the BM. Their results showed that cPC are a unique subpopulation of malignant PC in MM; cPC are characterized by decreased expression of integrins (CD11a/CD11c/CD29/CD49d/CD49e) and adhesion molecules (CD33/CD56/CD117/CD138). cPC were also mostly quiescent with higher clonogenic potential than BMPC [31].

Mishima et al. investigated genomic characterization of MM patients using cPC and wondered if mutational profile of cPC is in concordance with mutational profile of PC from the BM. This study showed that both populations have similar mutations. Interestingly, 100% of clonal mutations found in PC from BM were also detected in cPC. Moreover, 99% of clonal mutations of cPC were also found in BMPC. Whole genome sequencing did not find any major differences between these two groups of MM cells, suggesting that the change in biological behavior of cPC could be based on the changes of expression of ncRNA molecules [32].

3. Cell-free DNA

Cell-free DNA are short fragments of DNA not associated with a cell and found in PB and other body fluids, such as urine, saliva, breast milk and others [33–35]. The term cfDNA is a general term that includes circulating DNA of both healthy and tumor origin. As circulating tumor DNA (ctDNA) fragments represent only a fraction of total cfDNA, it is necessary to distinguish the origin of fragments during analysis. Physiological levels of cfDNA in PB of healthy individuals are generally low (10–100 ng/ml). This changes, however, in case of various pathological events. Elevated levels of cfDNA were described in patients with inflammation, trauma, sepsis, stroke or heart attack [36–38], but the highest levels of cfDNA were found in cancer patients, where they reached up to 1000 ng/ml [39, 40]. These findings suggest a correlation between levels of cfDNA and tumor burden. Nevertheless, cfDNA levels were not found to be cancer-specific. Stability of cfDNA is variable, ranging from 15 minutes to 2.5 hours; therefore, the amount of cfDNA cannot be used as a diagnostic marker [41].

Cells can release cfDNA either actively, using exosomes [42], or passively by apoptosis and necrosis (**Figure 1**) [43, 44]. In 2016, however, a study by Bronkhorst et al. proposed that apoptosis and necrosis are not the source of cfDNA and that active secretion is primarily used for cfDNA release [45]. This proposal will require further investigation as it suggests a more active role of cfDNA in cell-to-cell communication. Since cfDNA is released directly from cells, circulating fragments contain the same genetic information as the original cell. In case of cancer cells, this allows detection of cancer-specific genetic and epigenetic aberrations, such as mutations, microsatellite alterations, changes in DNA methylation and others [41].

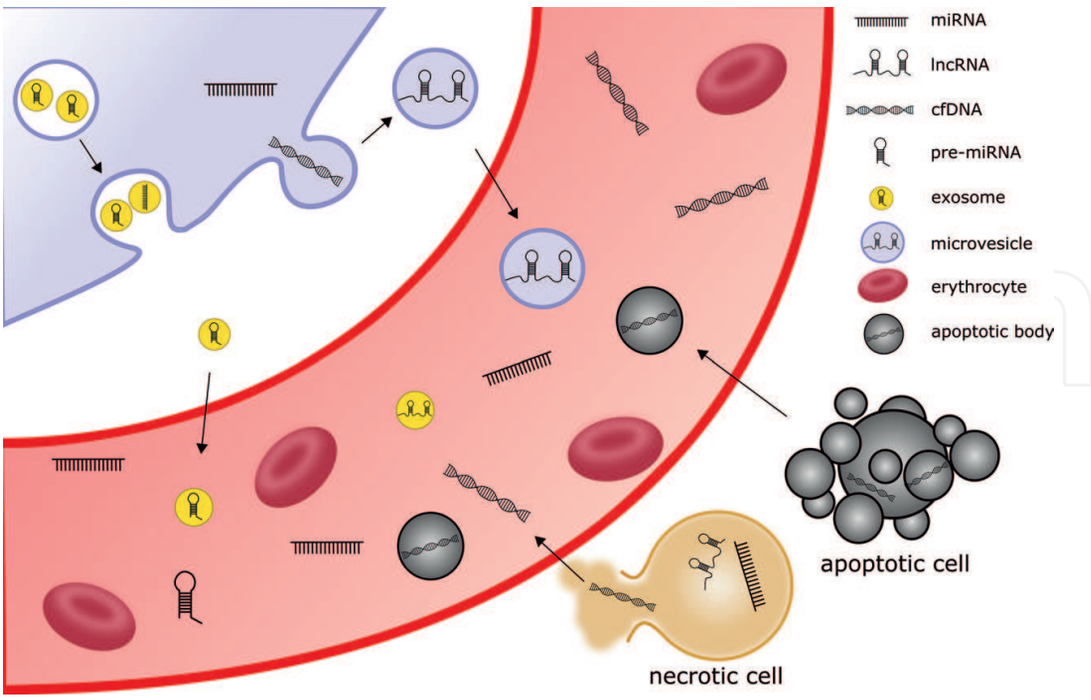

Figure 1. Schematic structure of release of circulating molecules and vesicles into bloodstream. miRNA – Micro RNA, lncRNA – Long non-coding RNA, cfDNA – Cell-free DNA and pre-miRNA – Precursor miRNA.

In the field of MM research, only a small number of cfDNA studies have been published so far. The first pilot study was published by Sata et al. In 2015 [23] they compared ASO-PCR data from peripheral blood mononuclear cells (PBMC), BM mononuclear cells (BMMC), CD20 + CD38− B-cell population in BM and serum cfDNA. Even though the study was quite small and only 20 patients (out of 30 enrolled) were quantifiable, it provided interesting results suggesting further studies and validation are needed. A strong correlation between BMMC and PBMC was found, suggesting circulation of clonogenic PC in PB; PBMC also negatively correlated with treatment as ASO-PCR data from those cells always decreased after treatment. These results suggest a possibility to use PBMC instead of BMMC in monitoring of MRD in MM patients. In addition, DNA sequences found in cfDNA were identical to those found in BM cells in 18/20 cases at diagnosis and 16/20 cases of follow-up samples, while levels of cfDNA remained mostly stable during the course of therapy. Based on these results, the authors assumed that detection of tumor V(D)J rearrangement in cfDNA can reflect presence and persistence of MM clones in patients. However, because of insufficient number of patients who reached complete remission (CR), the potential of cfDNA analysis for MRD monitoring remained unclear [46].

In 2017, three important studies on this topic were published [20, 47, 48]. The first by Kis et al. compared cfDNA analysis to BM analysis regarding molecular profiling of disease. This study screened 64 cfDNA samples from 53 MM patients for sequences of all protein-coding exons of *KRAS*, *NRAS*, *BRAF*, *EGFR* and *PIK3CA* genes. This method allowed for detection of tumor-related fragment of cfDNA at significantly low allele frequencies (0.25%). In 48 cfDNA samples, matching BM data were available. The analysis detected 49/51 (96%) of somatic mutations in cfDNA that were also found in BM; importantly, four additional mutations not detected in BM samples were found in cfDNA (>98% specificity). There were two mutations missed by sequencing of cfDNA samples that were detected during validation by ddPCR in BM samples but not in cfDNA. These outcomes emphasize the potential of cfDNA analysis not only for complex molecular profiling but also for detection of subclones not detected in BM aspirates [20].

The second important study, although once again lacking a larger patient cohort, was conducted by Oberle et al. and focused on detection of clonotypic V(D)J rearrangement in circulating MM cells and cfDNA. A cohort of 27 MM patients with various treatment regimens based on bortezomib, lenalidomide and panobinostat was examined. NGS was used for identification and tracking of patient-specific V(D)J rearrangements. The identification of rearrangements was successful in only 23 out of 27 patients, and these patients underwent further screening of blood samples before and after initiation of therapy. Baseline screening detected patient-specific V(D)J rearrangement in 71% of cases in circulating MM cells and in 100% of cases in cfDNA. However, these values decreased in follow-up samples to 40% and 34%, respectively. The results also correlated with remission status of patients—91% of non-responders/progressors and 41% of responders to therapy had evidence of persistent MM in circulating cells or cfDNA. Interestingly, positivity in circulating MM cells and cfDNA associated with each other ($P = 0.042$) but disagreed in 30% of cases. This suggests that circulating MM cells are not the only source of MM cfDNA and that cfDNA may reflect tumor burden more comprehensively. All these results indicate that V(D)J analysis from PB samples may be used for evaluation of treatment efficacy and possibly even for MRD prediction [48]. However, validation on a larger cohort is necessary.

The last study was published by Mithraprabhu et al., and the subject of this study was mutational characterization of MM. Paired DNA samples of BM PC and plasma derived cfDNA were analyzed for the presence of activating mutations of four oncogenes—*KRAS*, *NRAS*, *BRAF* and *TP53* by NGS. In total, 48 MM patients (33 relapsed/refractory and 15 newly diagnosed) and 21 healthy donors (HD) enrolled in the study. Overall, 128 different mutations were detected in MM patients (cfDNA = 31, BM = 59 and both = 38), while none were found in HD. Interestingly, almost a quarter of all found mutations were detected only in cfDNA samples. These findings proved spatial heterogeneity of MM and showed that cfDNA molecules are derived from multiple tumor sites within a patient's body. This was supported also by majority of cfDNA-specific mutations found in relapsed/refractory patients (30 mutations) in contrast to newly diagnosed patients (1 mutation) as they are more prone to have multiple focal lesions. Moreover, sequences of cfDNA were evaluated by ddPCR in seven patients throughout their treatment, and changes in fractional abundance were discovered, reflecting progression of disease. This proof-of-concept study confirmed the presence of mutations only in cfDNA and proved that genetic composition of MM is complex and evolves during progression of disease. In addition, it proposed that cfDNA analysis could be used as an adjunct to standard BM biopsy for disease monitoring to enable obtaining more complex results [47].

So far, not many cfDNA studies in MM have been conducted; however, the data are exciting and strongly suggest the future role of cfDNA in MRD monitoring.

4. Non-coding RNA molecules

Protein-coding genes comprise only about 1.5% of the genome. At the same time, it was shown that more than 90% is transcriptionally active [49]. Transcription of this so-called junk DNA leads to creation of thousands of RNA molecules that are not capable of coding proteins. Surprisingly, it was shown that the more complex the organism, the higher number of non-coding RNA (ncRNA) molecules it contains [50]. These molecules have many different functions in the most important cell processes, such as differentiation, proliferation, apoptosis and many others. They are involved in tumorigenesis as well.

Based on their length, ncRNA are divided into two groups: short (sncRNA) and long (lncRNA). SncRNA are smaller than 200 nucleotides (nt), while lncRNA are longer than 200 nt. The first ncRNA molecules that were identified more than 50 years ago were ribosomal RNA (rRNA) and transfer RNA (tRNA) [4, 5, 51]. While there are many classes of ncRNA, the most studied and well known are microRNA (miRNA) and long non-coding RNA (lncRNA).

4.1. Definition and biogenesis of microRNA

MiRNA are short, non-coding, single-stranded RNA molecules about 21–23 nt long. They are involved in regulation of gene expression and influence various cell processes, such as proliferation, differentiation, apoptosis and tumorigenesis. MiRNA genes account for 1–2% of the human genome, and mature miRNA regulate around 50% of protein-coding genes [49].

Based on the canonical model of miRNA biogenesis, miRNA genes are transcribed by RNA polymerase II or III into primary precursors, stem-loop structures (pri-miRNA) that contain 5′ end cap and polyA on the 3′ end. Pri-miRNA are cleaved in the nucleus by RNAse II enzyme Drosha and Pasha leading to pre-miRNA [52]. Pre-miRNA are exported into cytoplasm by transport protein exportin 5 [53]. In the cytoplasm, the pre-miRNA molecule is processed by the RISC complex that contains RNAse III Dicer and protein Argonaute 2 (Ago2). RISC complex cuts the molecule into 20–23 nt long double-stranded miRNA duplex with 2 nt overhang on 3′ ends [54]. One of the strands is the so-called guide strand and is complementary to the mRNA sequence. The other ('passenger' strand) is degraded. Which one of these strands is degraded is based on the stability of pairing on the 5′ end of the miRNA duplex [55]. Based on the level of miRNA/mRNA complementarity, the target mRNA is either silenced translationally in case of non-complete complementarity or degraded in case of 100% complementarity [56, 57].

MiRNA regulate a large spectrum of physiological and pathological processes including oncogenesis; they can act as oncogenes or tumor suppressors. Several mechanisms of miRNA role in tumorigenesis have been described: increased expression levels, amplification, epigenetic silencing or loss of miRNA gene that regulates expression of a tumor suppressor gene [58]. On the other hand, deletion and epigenetic silencing of miRNA gene expression that silences oncogene expression have been described as well [59]. Moreover, mutations in target sequences of mRNA lead to failed translational repression or degradation of target mRNA [60].

In a pilot study of miRNA expression in malignant transformation of PC, increased expression of miR-181a/b, cluster miR-106b-25 (miR-93, miR-106b, miR 25) and miR-21 in MGUS and MM patients in comparison to healthy donors (HD) was found. Interestingly, MM patients showed increased expression of cluster miR-17-92a suggesting a possible role of this cluster in disease progression [61].

4.2. Circulating miRNA

Essentially, all human body fluids (PB, saliva, urine, breast milk, etc.) contain the so-called circulating miRNA [62]. Circulating miRNA are quite stable and resistant to RNases as they are part of protein (Ago2) or lipoprotein (high-density lipoprotein (HDL)) complexes or they are bound inside exosomes—small transport vesicles [63]. It seems that circulating miRNA are involved in cell-to-cell communication as they are exported outside of cells based on biological stimuli. These molecules can also take part in cell processes, such as communication, proliferation, differentiation and in case of tumors also metastases [64]. Specific profiles of circulating miRNA are diagnostic markers differentiating HD from patients, but they also correlate with progression and staging of the tumor [65, 66]. A major advantage of these molecules as potential biomarkers is their simple structure, easy access and measurability by standard laboratory techniques [64].

4.2.1. Circulating microRNA in monoclonal gammopathies

In MM, circulating miRNA were first described in 2012. In a study by Jones et al., PB serum samples of MGUS and MM patients were analyzed in comparison to HD. They found that miR-720, miR-1246 and miR-1308 could serve as potential markers of MG [67].

The success of this study led to other studies, especially in MM. However, different approaches lead to varying results. The main differences were type of samples (serum or plasma of PB), design of experiments (patients vs. HD) and used methods and platforms.

Plasma of PB was reported to have lower levels of miR-92a in newly diagnosed MM patients in comparison to HD. Moreover, the level of miR-92a fluctuated based on progression of disease and treatment response, which would suggest a possible role of this miRNA as a predictive biomarker [68]. Another study showed increased expression of miR-148a, miR-181a, miR-20a, miR-221 and miR-88b in plasma of PB of MM patients in comparison with HD. Expression level of miR-20a and miR-148a was connected to shorter time to relapse of MM; this study suggested that circulating plasma miR-20a could be a marker of worse prognosis of MM [69]. On contrary, another study showed mostly decreased miRNA expression in MM patients compared to HD. MiR-483-5p and miR-20a were shown to have diagnostic and prognostic potential [70].

So far, most studies were performed using serum miRNA. Our own pilot study showed significantly increased levels of miR-29a, miR-660 and miR-142-5p in MM patients in comparison with HD. We showed that circulating serum miR-29a could be a biomarker for MM patients [71]. In our follow-up study, we showed dysregulation of five serum miRNA, miR-744, miR-130a, let-7d, let-7e and miR-34a in MGUS and MM patients in comparison with HD. Multivariate analysis showed that combination of miR-34a and let-7e distinguishes the patient cohorts with good sensitivity and specificity. Moreover, dynamics of serum miRNA with disease progression was shown [72].

In another study, increased expression of miR-181a/b, miR-221, miR-222 and miR-382 was found in relapsed MM patients and MM cell lines [51]. On contrary, lower expression of miR-15a and miR-16 was described; these miRNA have been described in chronic lymphocytic leukemia and seem to be part of pathogenesis of this disease. The genes for these miRNA are coded in the 13q14 locus; this locus is often deleted also in MM [73]. MiR-15a and miR-16 support apoptosis and decrease proliferation of MM cells by AKT and MAP kinase signaling [51].

In a study by Rocci et al., higher levels of miR-25, miR-16 and miR-30a in MM patients correlated with longer overall survival (OS) [74]. Another study showed that miR-19a and miR-4254 distinguish MM and HD. In addition, decreased level of serum miR-19a was positively correlated with international staging system (ISS) stage, presence of del(13q14) and gain 1q21 and shorter progression-free survival (PFS) and OS. Surprisingly, these patients responded to bortezomib better [75].

Serum miRNA were also analyzed at CR after autologous stem cell transplantation (ASCT). MiR-16, miR-17, miR-19b, miR-20a and miR-660 were decreased in diagnostic samples in comparison with CR samples [76]. Patients with lower levels of miR-19b and miR-331 had shorter PFS after ASCT. Level of miR-19b was significantly lower in samples obtained at relapsed than at CR.

The most common clinical manifestation of MM is osteolytic lesions. Increased levels of serum miR-214 and miR-135b were found in MM patients with osteolytic lesions, and their expression correlated with severity of the symptoms [77]. Moreover, increased level of miR-214 associated with shorter PFS and OS.

Using NGS, miRNA (let-7b a miR-18a) from exosomes isolated from serum of MM patients significantly correlated with PFS and OS in univariate analysis and with ISS and cytogenetic abnormalities in multivariate analysis [78]. Moreover, it was shown that levels of exosomal

miR-16-5p, miR-15a-5p, miR-20a-5p and miR-17-5p were significantly decreased in MM patients resistant to bortezomib [79].

As for miRNA in other body fluids, our group performed analysis of circulating miRNA in urine of MM patients in comparison with HD. Unfortunately, we did not find any miRNA significantly dysregulated [13].

While a lot of work was done on circulating miRNA in MG, so far no clear biomarkers of the diseases have been identified. It is possible that MM as a heterogeneous disease will not have a single circulating miRNA as a biomarker. Further studies and standardization of sample processing, types of samples and analytical methods need to be performed.

In our opinion, miRNA have a large potential for diagnostics of monoclonal gammopathies. Most studies were done on diagnostics samples; however, the data are not consistent and more standardization and optimization is needed. The possibility of using miRNA as prognostic or monitoring markers needs to be further validated.

4.3. Long non-coding RNA molecules

LncRNA are an abundant class of RNA between 200 nt and 100 kb long [80, 81]. To date, approximately 16,000 lncRNA have been identified in the human genome (http://www.gencodegenes.org/). On the other hand, the functional characterization of most of them has not been determined yet.

Genes encoding for lncRNA are present in many types of organisms, including animals [82], plants [83], yeast [84], prokaryotic organisms [85] and viruses [86]. LncRNA do not possess protein-coding capacity due to the absence of open reading frames (ORFs) or because of insufficient length of ORFs [87–90]. They can be classified according to their genomic localization into three major groups: long intergenic non-coding RNA (lincRNA), long intronic RNA and long non-coding RNA transcribed from specific genomic regions.

The expression of lncRNA genes is developmental and tissue-specific, and they have been associated with a large spectrum of biological processes, for example, alternative splicing, modulation of protein activity, alternation of protein localization, epigenetic regulation and generally regulation of gene expression. These molecules can be precursors of small RNA and even tools for miRNA silencing [91–96]. LncRNA play an important role both in physiological and pathological processes. The deregulated expression levels of these molecules were identified in a large variety of tumor diseases: breast cancer [97], small-cell lung carcinoma [98] and also in MM [22]. It was shown that alterations in lncRNA can influence regulation of cancer progression [99]. Interestingly, lncRNA seem to have higher tissue specificity even in comparison with protein-coding mRNA and miRNA. Thus, they are even more interesting as new specific biomarkers [88].

Function of lncRNA can also be derived from their localization within the cell. These molecules can be found in the nucleus and in the cytoplasm. LncRNA are preferably located in the cell nucleus, deriving their significant effect on the DNA sequence [88].

The classification of lncRNA can be based on their influence on the DNA sequence. From this perspective, there are two classes of lncRNA: cis-lncRNA (cis-acting lncRNA) and trans-lncRNA (trans-acting lncRNA). Cis-IncRNA can positively or negatively regulate expression

of genes that are located in their genomic proximity [95]. On the other hand, trans-lncRNA regulate expression of distant genes [100]. Many lncRNA are transcribed by RNA polymerase II, just like protein-coding genes. If lncRNA are involved in the regulation of RNA polymerase II, they are transcribed by RNA polymerase III [87, 101–103]. High degree of evolutionary conservation, tissue-specific expression and stability of lncRNA point to significant functional role of these molecules [104].

Dysregulation of lncRNA expression was observed in various human diseases, including cancer. LncRNA may be either oncogenes or tumor suppressors in development as well as progression of tumors [105, 106]. Changes of expression levels of several lncRNA have been reported in several malignancies; other lncRNA seem to be specific for a single tumor, suggesting that these molecules may be good biomarkers for tumor diagnostics as well as prognosis and prediction [107].

Moreover, it was shown that lncRNA are involved in regulation of hematopoiesis, including proliferation, differentiation and apoptosis of hematopoietic stem cells as well as progenitors and precursors of mature blood cells [108, 109]. Dysregulated expression of lncRNA was reported in lymphomas, leukemias and MM. It seems possible that expression profile of these lncRNA could have a potential clinical significance in diagnostics and prognostics of hematologic malignancies.

Current information about the role of lncRNA in pathogenesis of MM is very limited. So far, MALAT1 has been described as a marker of early progression [110]. Expression level of this lncRNA was increased in BM cells of newly diagnosed MM patients and changed during progression of the disease. Patients with lower levels of MALAT1 had a higher risk of early progression.

Handa et al. showed higher expression level of MALAT1 in MM patients in comparison to MGUS and HD [111]. These results are in correlation with another study of Ronchetti et al. who showed dysregulation of 31 lncRNA, including MALAT1, in MM patients [112]. Moreover, this lncRNA may be important in MM pathogenesis through activation of TGF-β, a factor important for osteolytic lesion formation [113].

An earlier study showed decreased expression of MEG3 in MM patients [114]. Decreased expression or loss of this lncRNA seems to be important in various types of human tumors [115]. In a study by Benetatos et al., MEG3 was reported to be lost in more than half of MM patients, and it seemed to have a prognostic significance for MM [116].

Our own study showed that UCA1 might be a marker of MM when HD, MGUS and MM plasma cells were compared (with sensitivity of 85.0% and specificity of 94.7%). UCA1 levels seemed to correlate with albumin and monoclonal immunoglobulin serum levels, cytogenetic aberrations, and survival of MM patients.

4.3.1. Circulating lncRNA

Similar to circulating miRNA, even lncRNA may be detected in body fluids suggesting their possible role as biomarkers for diagnosis, prognosis and prediction. They were found in PB

and urine, but they can also be found within exosomes where they are protected against RNases [117].

Most studies of circulating lncRNA published so far were studies of solid tumors. In prostate cancer, PCA3 specificity was so high that a new test from urine has been approved for usage in Europe; it can be used together with currently used PSA test (prostate-specific antigen) [118–121].

In urinary bladder cancer, increased level of UCA1 was detected not only in the tumor tissue but also in PB and urine of patients [79, 122]. It was shown that based on UCA1 expression, urinary bladder cancer may be distinguished from other urinary bladder diseases with high specificity [28].

Unfortunately, only very few studies were published about circulating lncRNA in MM. In a study of Isin et al. [123], five candidate lncRNA (TUG1, MALAT1, HOTAIR, GAS5, lincRNA-p21) were analyzed in plasma of PB of MM patients in comparison with CLL patients [103]. Plasma of PB of CLL patients contained significantly deregulated levels of lincRNA-p21. On the other hand, MM plasma contained deregulated levels of the other four lncRNA. When compared to HD, MM patients contained only TUG1 deregulated levels. There was a correlation of circulating lncRNA and clinical subgroups of MM, suggesting that TU1 could be a part of MM progression. Another study reported significantly higher levels of PCAT-1 in MM patients in comparison with HD. Its potential as a biomarker was proven by ROC analysis that showed sensitivity of 71.7% and specificity of 93.8%. A possible correlation with MM pathogenesis was suggested by a correlation with β2 microglobulin [123].

While lncRNA molecules are generally described as being more tissue-specific than miRNA, not enough data have been published on circulating lncRNA in MM. Further studies that are more comprehensive are needed to verify their claim as the more specific marker.

5. Conclusion

While not many studies have been published dealing with liquid biopsies of circulating molecules in multiple myeloma, they show a great promise. Liquid biopsies could be used as an adjunct to standard BM biopsy for disease monitoring to enable obtaining more complex results and easier follow-up of patients. While there are many candidate molecules that have been described in this review (cfDNA, miRNA and lncRNA), more studies are needed to validate these findings.

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References

[1] Calame KL. Plasma cells: Finding new light at the end of B cell development. Nature Immunology. 2001;**2**(12):1103-1108

[2] International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: A report of the international myeloma working group. British Journal of Haematology. 2003;**121**(5):749-757

[3] Roccaro AM, Ghobrial IM, editors. In: Plasma Cell Dyscrasias. Springer International Publishing; 2016. (Cancer Treatment and Research). Available from: //www.springer.com/la/book/9783319403182

[4] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a Cancer Journal for Clinicians. 2016;**66**(1):7-30

[5] Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos M-V, et al. Multiple myeloma. Nature Reviews Disease Primers. 2017;**3**:17046

[6] Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos M-V, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. The Lancet Oncology. 2014;**15**(12):e538-e548

[7] Maluskova D, Svobodová I, Kucerova M, Brozova L, Muzik J, Jarkovský J, et al. Epidemiology of multiple myeloma in the Czech Republic. Klin Onkol Cas Ceské Slov Onkol Spolecnosti. 2017;**30**(Suppl. 2):35-42

[8] Dimopoulos MA, Terpos E. Multiple myeloma. Annals of Oncology, the journal of the European Society for Medical Oncology. 2010;**21**(Suppl 7):vii143-vii150

[9] Becker N. Epidemiology of multiple myeloma. Recent Results in Cancer Research. 2011;**183**:25-35

[10] Rajkumar SV. Updated diagnostic criteria and staging system for multiple myeloma. American Society of Clinical Oncology Educational Book. 2016;**35**:e418-e423

[11] Flores-Montero J, Sanoja-Flores L, Paiva B, Puig N, García-Sánchez O, Böttcher S, et al. Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia. 2017;**31**(10):2094-2103

[12] Rajkumar SV, Harousseau J-L, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al. Consensus recommendations for the uniform reporting of clinical trials: Report of the international myeloma workshop consensus panel 1. Blood. 2011;**117**(18):4691-4695

[13] Sedlarikova L, Kubiczkova L, Kryukov F, Pelcova J, Adam Z, Pour L, et al. Detection of tumor-specific marker for minimal residual disease in multiple myeloma patients. Biomedical Papers of the Medical Faculty of the University Palacký, Olomouc, Czechoslovakia. 2015;**159**(4):554-561

[14] Furukawa Y, Kikuchi J. Molecular pathogenesis of multiple myeloma. International Journal of Clinical Oncology. 2015;**20**(3):413-422

[15] Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003;**101**(11):4569-4575

[16] Rasche L, Chavan SS, Stephens OW, Patel PH, Tytarenko R, Ashby C, et al. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. Nature Communications. 2017;**8**(1):268

[17] Weinhold N, Chavan SS, Heuck C, Stephens OW, Tytarenko R, Bauer M, et al. High risk multiple myeloma demonstrates marked spatial genomic heterogeneity between focal lesions and random bone marrow; implications for targeted therapy and treatment resistance. Blood. 2015;**126**:20-20

[18] Kubaczkova V, Vrabel D, Sedlarikova L, Besse L, Sevcikova S. Cell-free DNA - minimally invasive marker of hematological malignancies. European Journal of Haematology. 2017;**99**(4):291-299

[19] Qasaimeh MA, Wu YC, Bose S, Menachery A, Talluri S, Gonzalez G, et al. Isolation of circulating plasma cells in multiple myeloma using CD138 antibody-based capture in a microfluidic device. Scientific Reports. 2017;**7**:45681; 04 12/14/received 03/02/accepted

[20] Kis O, Kaedbey R, Chow S, Danesh A, Dowar M, Li T, et al. Circulating tumour DNA sequence analysis as an alternative to multiple myeloma bone marrow aspirates. Nature Communications. 2017;**8**:15086

[21] Besse L, Sedlarikova L, Kryukov F, Nekvindova J, Radova L, Slaby O, et al. Circulating serum microRNA-130a as a novel putative marker of extramedullary myeloma. PLoS One. 2015;**10**(9):e0137294

[22] Sedlarikova L, Gromesova B, Kubaczkova V, Radova L, Filipova J, Jarkovsky J, et al. Deregulated expression of long non-coding RNA UCA1 in multiple myeloma. European Journal of Haematology. 2017;**99**(3):223-233

[23] Billadeau D, Van Ness B, Kimlinger T, Kyle RA, Therneau TM, Greipp PR, et al. Clonal circulating cells are common in plasma cell proliferative disorders: A comparison of monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, and active myeloma. Blood. 1996;**88**(1):289-296

[24] Nowakowski GS, Witzig TE, Dingli D, Tracz MJ, Gertz MA, Lacy MQ, et al. Circulating plasma cells detected by flow cytometry as a predictor of survival in 302 patients with newly diagnosed multiple myeloma. Blood. 2005;**106**(7):2276-2279

[25] Dingli D, Nowakowski GS, Dispenzieri A, Lacy MQ, Hayman SR, Rajkumar SV, et al. Flow cytometric detection of circulating myeloma cells before transplantation in patients with multiple myeloma: A simple risk stratification system. Blood. 2006;**107**(8):3384-3388

[26] Kumar S, Witzig TE, Greipp PR, Rajkumar SV. Bone marrow angiogenesis and circulating plasma cells in multiple myeloma. British Journal of Haematology. 2003;**122**(2):272-274

[27] Pour L, Sevcikova S, Greslikova H, Kupska R, Majkova P, Zahradova L, et al. Soft-tissue extramedullary multiple myeloma prognosis is significantly worse in comparison to bone-related extramedullary relapse. Haematologica. 2014;**99**(2):360-364

[28] Granell M, Calvo X, Garcia-Guiñón A, Escoda L, Abella E, Martínez CM, et al. Prognostic impact of circulating plasma cells in patients with multiple myeloma: Implications for plasma cell leukemia definition. Haematologica. 2017;**102**(6):1099-1104

[29] San Miguel JF, Gutiérrez NC, Mateo G, Orfao A. Conventional diagnostics in multiple myeloma. European Journal of Cancer (Oxford, England: 1990). 2006;**42**(11):1510-1519

[30] Chaidos A, Barnes CP, Cowan G, May PC, Melo V, Hatjiharissi E, et al. Clinical drug resistance linked to interconvertible phenotypic and functional states of tumor-propagating cells in multiple myeloma. Blood. 2013;**121**(2):318-328

[31] Paiva B, Paino T, Sayagues J-M, Garayoa M, San-Segundo L, Martín M, et al. Detailed characterization of multiple myeloma circulating tumor cells shows unique phenotypic, cytogenetic, functional, and circadian distribution profile. Blood. 2013;**122**(22):3591-3598

[32] Mishima Y, Paiva B, Shi J, Park J, Manier S, Takagi S, et al. The mutational landscape of circulating tumor cells in multiple myeloma. Cell Reports. 2017;**19**(1):218-224

[33] Bryzgunova OE, Skvortsova TE, Kolesnikova EV, Starikov AV, Rykova EY, Vlassov VV, et al. Isolation and comparative study of cell-free nucleic acids from human urine. Annals of the New York Academy of Sciences. 2006;**1075**:334-340

[34] Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Research. 2001;**61**(4):1659-1665

[35] Volik S, Alcaide M, Morin RD, Collins C. Cell-free DNA (cfDNA): Clinical significance and utility in cancer shaped by emerging technologies. Molecular Cancer Research. 2016;**14**:898-908

[36] Dwivedi DJ, Toltl LJ, Swystun LL, Pogue J, Liaw K-L, Weitz JI, et al. Prognostic utility and characterization of cell-free DNA in patients with severe sepsis. Critical Care (London, England). 2012;**16**(4):R151

[37] Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. Clinical Chemistry. 2000;**46**(3):319-323

[38] Rainer TH, Wong LKS, Lam W, Yuen E, Lam NYL, Metreweli C, et al. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. Clinical Chemistry. 2003;**49**(4):562-569

[39] Basnet S, Zhang ZY, Liao WQ, Li SH, Li PS, Ge HY. The prognostic value of circulating cell-free DNA in colorectal cancer: A meta-analysis. Journal of Cancer. 2016;**7**:1105-1113

[40] Chun FK-H, Müller I, Lange I, Friedrich MG, Erbersdobler A, Karakiewicz PI, et al. Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer. BJU International. 2006;**98**(3):544-548

[41] Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer--a survey. Biochimica et Biophysica Acta. 2007;**1775**(1):181-232

[42] Wang W, Kong P, Ma G, Li L, Zhu J, Xia T, et al. Characterization of the release and biological significance of cell-free DNA from breast cancer cell lines. Oncotarget. 2017;**8**(26):43180-43191

[43] Elshimali YI, Khaddour H, Sarkissyan M, Wu Y, Vadgama JV. The clinical utilization of circulating cell free DNA (CCFDNA) in blood of cancer patients. International Journal of Molecular Sciences. 2013;**14**(9):18925-18958

[44] Salvi S, Gurioli G, De Giorgi U, Conteduca V, Tedaldi G, Calistri D, et al. Cell-free DNA as a diagnostic marker for cancer: Current insights. OncoTargets and Therapy. 2016;**9**:6549-6559

[45] Bronkhorst AJ, Wentzel JF, Aucamp J, van Dyk E, du Plessis L, Pretorius PJ. Characterization of the cell-free DNA released by cultured cancer cells. Biochimica et Biophysica Acta. 2016;**1863**(1):157-165

[46] Sata H, Shibayama H, Maeda I, Habuchi Y, Nakatani E, Fukushima K, et al. Quantitative polymerase chain reaction analysis with allele-specific oligonucleotide primers for individual IgH VDJ regions to evaluate tumor burden in myeloma patients. Experimental Hematology. 2015;**43**(5):374-381.e2

[47] Mithraprabhu S, Khong T, Ramachandran M, Chow A, Klarica D, Mai L, et al. Circulating tumour DNA analysis demonstrates spatial mutational heterogeneity that coincides with disease relapse in myeloma. Leukemia. 2017;**31**(8):1695-1705

[48] Oberle A, Brandt A, Voigtlaender M, Thiele B, Radloff J, Schulenkorf A, et al. Monitoring multiple myeloma by next-generation sequencing of V(D)J rearrangements from circulating myeloma cells and cell-free myeloma DNA. Haematologica. 2017;**102**(6): 1105-1111

[49] Sana J, Faltejskova P, Svoboda M, Slaby O. Novel classes of non-coding RNAs and cancer. Journal of Translational Medicine. 2012;**10**:103

[50] Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: Regulators of disease. The Journal of Pathology. 2010;**220**(2):126-139

[51] Roccaro AM, Sacco A, Thompson B, Leleu X, Azab AK, Azab F, et al. MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma. Blood. 2009;**113**(26):6669-6680

[52] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;**425**(6956):415-419

[53] Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes & Development. 2003;**17**(24):3011-3016

[54] Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes & Development. 2001;**15**(20):2654-2659

[55] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nature Reviews. Cancer. 2006;**6**(4):259-269

[56] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nature Reviews. Cancer. 2015;**15**(6):321-333

[57] Slabý O, Svoboda M, Bešše A, Bienertová Vašků J, Černá K, Doležalová D, et al. MikroRNA v onkologii [Internet]. Galén; 2012 [cited 2018 Jun 4]. Available from: https://is.muni.cz/repo/987454/

[58] He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature. 2005;**435**(7043):828-833

[59] Shi Y, Huang J, Zhou J, Liu Y, Fu X, Li Y, et al. MicroRNA-204 inhibits proliferation, migration, invasion and epithelial-mesenchymal transition in osteosarcoma cells via targeting Sirtuin 1. Oncology Reports. 2015;**34**(1, 1):399-406

[60] Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science. 2007;**315**(5818):1576-1579

[61] Pichiorri F, Suh S-S, Ladetto M, Kuehl M, Palumbo T, Drandi D, et al. MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(35):12885-12890

[62] Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clinical Chemistry. 2010;**56**(11):1733-1741

[63] Esteller M. Non-coding RNAs in human disease. Nature Reviews. Genetics. 2011;**12**(12):861-874

[64] Wang K, Yuan Y, Cho J-H, McClarty S, Baxter D, Galas DJ. Comparing the MicroRNA Spectrum between serum and plasma. PLoS One. 2012;**7**(7):e41561

[65] Menéndez P, Villarejo P, Padilla D, Menéndez JM, Montes JAR. Diagnostic and prognostic significance of serum MicroRNAs in colorectal cancer. Journal of Surgical Oncology. 2013;**107**(2):217-220

[66] Mitchell JS, Li N, Weinhold N, Försti A, Ali M, van Duin M, et al. Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. Nature Communications. 2016;**7**:12050

[67] Jones CI, Zabolotskaya MV, King AJ, Stewart HJS, Horne GA, Chevassut TJ, et al. Identification of circulating microRNAs as diagnostic biomarkers for use in multiple myeloma. British Journal of Cancer. 2012;**107**(12):1987-1996

[68] Yoshizawa S, Ohyashiki JH, Ohyashiki M, Umezu T, Suzuki K, Inagaki A, et al. Downregulated plasma miR-92a levels have clinical impact on multiple myeloma and related disorders. Blood Cancer Journal. 2012;**2**(1):e53

[69] Huang J, Yu J, Li J, Liu Y, Zhong R. Circulating microRNA expression is associated with genetic subtype and survival of multiple myeloma. Medical oncology (Northwood, London, England). 2012;**29**(4):2402-2408

[70] Qu X, Zhao M, Wu S, Yu W, Xu J, Xu J, et al. Circulating microRNA 483-5p as a novel biomarker for diagnosis survival prediction in multiple myeloma. Medical oncology (Northwood, London, England). 2014;**31**(10):219-219

[71] Sevcikova S, Kubiczkova L, Sedlarikova L, Slaby O, Hajek R. Serum miR-29a as a marker of multiple myeloma. Leukemia & Lymphoma. 2013;**54**(1):189-191

[72] Kubiczkova L, Pour L, Sedlarikova L, Hajek R, Sevcikova S. Proteasome inhibitors - molecular basis and current perspectives in multiple myeloma. Journal of Cellular and Molecular Medicine. 2014;**18**(6):947-961

[73] Walker BA, Leone PE, Chiecchio L, Dickens NJ, Jenner MW, Boyd KD, et al. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. Blood. 2010;**116**(15):e56-e65

[74] Rocci A, Hofmeister CC, Geyer S, Stiff A, Gambella M, Cascione L, et al. Circulating miRNA markers show promise as new prognosticators for multiple myeloma. Leukemia. 2014;**28**(9):1922-1926

[75] Hao M, Zang M, Wendlandt E, Xu Y, An G, Gong D, et al. Low serum miR-19a expression as a novel poor prognostic indicator in multiple myeloma. International Journal of Cancer. 2015;**136**(8):1835-1844

[76] Navarro A, Díaz T, Tovar N, Pedrosa F, Tejero R, Cibeira MT, et al. A serum microRNA signature associated with complete remission and progression after autologous stem-cell transplantation in patients with multiple myeloma. Oncotarget. 2015;**6**(3): 1874-1883

[77] Hao M, Zang M, Zhao L, Deng S, Xu Y, Qi F, et al. Serum high expression of miR-214 and miR-135b as novel predictor for myeloma bone disease development and prognosis. Oncotarget. 2016;**7**(15):19589-19600

[78] Manier S, Kawano Y, Bianchi G, Roccaro AM, Ghobrial IM. Cell autonomous and microenvironmental regulation of tumor progression in precursor states of multiple myeloma. Current Opinion in Hematology. 2016;**23**(4):426-433

[79] Zhang L, Pan L, Xiang B, Zhu H, Wu Y, Chen M, et al. Potential role of exosome-associated microRNA panels and in vivo environment to predict drug resistance for patients with multiple myeloma. Oncotarget. 2016;**7**(21):30876-30891

[80] Geisler S, Coller J. RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nature Reviews. Molecular Cell Biology. 2013;**14**(11):699-712

[81] Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nature Reviews. Genetics. 2016;**17**(1):47-62

[82] Brown CJ, Hendrich BD, Rupert JL, Lafrenière RG, Xing Y, Lawrence J, et al. The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell. 1992;**71**(3):527-542

[83] Swiezewski S, Liu F, Magusin A, Dean C. Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature. 2009;**462**(7274):799-802

[84] Houseley J, Rubbi L, Grunstein M, Tollervey D, Vogelauer M. A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. Molecular Cell. 2008;**32**(5, 5):685-695

[85] Bernstein E, Allis CD. RNA meets chromatin. Genes & Development. 2005;**19**(14):1635-1655

[86] Reeves MB, Davies AA, McSharry BP, Wilkinson GW, Sinclair JH. Complex I binding by a virally encoded RNA regulates mitochondria-induced cell death. Science. 2007;**316**(5829):1345-1348

[87] Calore F, Lovat F, Garofalo M. Non-coding RNAs and cancer. International Journal of Molecular Sciences. 2013;**14**(8):17085-17110

[88] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Research. 2012;**22**(9):1775-1789

[89] Knauss JL, Sun T. Regulatory mechanisms of long noncoding RNAs in vertebrate central nervous system development and function. Neuroscience. 2013;**235**:200-214

[90] Maruyama R, Suzuki H. Long noncoding RNA involvement in cancer. BMB Reports. 2012;**45**(11):604-611

[91] Chen Y, Song Y, Wang Z, Yue Z, Xu H, Xing C, et al. Altered expression of MiR-148a and MiR-152 in gastrointestinal cancers and its clinical significance. Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract. 2010;**14**(7):1170-1179

[92] Costa FF. Non-coding RNAs: Meet thy masters. BioEssays. 2010;**32**(7):599-608

[93] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010;**464**(7291):1071-1076

[94] Lipovich L, Johnson R, Lin C-Y. MacroRNA underdogs in a microRNA world: Evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. Biochimica et Biophysica Acta. 2010;**1799**(9):597-615

[95] Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, et al. Long noncoding RNAs with enhancer-like function in human cells. Cell. 2010;**143**(1, 1):46-58

[96] Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Molecular Cell. 2010;**39**(6):925-938

[97] Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. PLoS Genetics. 2010;**6**(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2996334/

[98] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, et al. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene. 2003;**22**(39):8031-8041

[99] Tano K, Akimitsu N. Long non-coding RNAs in cancer progression. Frontiers in Genetics. 2012;**3**:219

[100] Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biology 2013;**10**(6):924-933

[101] Alvarez-Dominguez JR, Hu W, Gromatzky AA, Lodish HF. Long noncoding RNAs during normal and malignant hematopoiesis. International Journal of Hematology. 2014;**99**(5):531-541

[102] Ayers D. Long non-coding RNAs: Novel emergent biomarkers for cancer diagnostics. Journal of Cancer Treatment and Research. 2013;**1**(2):31-35

[103] Morceau F, Chateauvieux S, Gaigneaux A, Dicato M, Diederich M. Long and short noncoding RNAs as regulators of hematopoietic differentiation. International Journal of Molecular Sciences. 2013;**14**(7):14744-14770

[104] Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biology. 2013;**10**(6):925-933

[105] Bartonicek N, Maag JLV, Dinger ME. Long noncoding RNAs in cancer: Mechanisms of action and technological advancements. Molecular Cancer. 2016;**15**:43

[106] Flippot R, Malouf GG, Su X, Mouawad R, Spano J-P, Khayat D. Cancer subtypes classification using long non-coding RNA. Oncotarget. 2016;**7**(33):54082-54093

[107] Gibb EA, Vucic EA, Enfield KSS, Stewart GL, Lonergan KM, Kennett JY, et al. Human cancer long non-coding RNA Transcriptomes. PLoS One. 2011;**6**(10):e25915

[108] Nobili L, Lionetti M, Neri A. Long non-coding RNAs in normal and malignant hematopoiesis. Oncotarget. 2016;**7**(31):50666-50681

[109] Wei P, Han B, Chen Y. Role of long non-coding RNAs in normal and malignant hematopoiesis. Science China. Life Sciences. 2013;**56**(10):867-875

[110] Cho S-F, Chang YC, Chang C-S, Lin S-F, Liu Y-C, Hsiao H-H, et al. MALAT1 long non-coding RNA is overexpressed in multiple myeloma and may serve as a marker to predict disease progression. BMC Cancer. 2014;**14**:809

[111] Handa H, Kuroda Y, Kimura K, Masuda Y, Hattori H, Alkebsi L, et al. Long non-coding RNA MALAT1 is an inducible stress response gene associated with extramedullary spread and poor prognosis of multiple myeloma. British Journal of Haematology. 2017;**179**(3):449-460

[112] Ronchetti D, Manzoni M, Todoerti K, Neri A, Agnelli L. In silico characterization of miRNA and long non-coding RNA interplay in multiple myeloma. Genes. 2016;**7**(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5192483/

[113] Li B, Chen P, Qu J, Shi L, Zhuang W, Fu J, et al. Activation of LTBP3 gene by a long non-coding RNA (lncRNA) MALAT1 transcript in mesenchymal stem cells from multiple myeloma. The Journal of Biological Chemistry. 2014;**289**(42):29365-29375

[114] Zhuang W, Ge X, Yang S, Huang M, Zhuang W, Chen P, et al. Upregulation of lncRNA MEG3 promotes osteogenic differentiation of mesenchymal stem cells from multiple myeloma patients by targeting BMP4 transcription. Stem Cells. 2015;**33**(6):1985-1997

[115] Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: A tumor suppressor. Journal of Molecular Endocrinology. 2012;**48**(3):R45-R53

[116] Benetatos L, Vartholomatos G, Hatzimichael E. MEG3 imprinted gene contribution in tumorigenesis. International Journal of Cancer. 2011;**129**(4):773-779

[117] Qi P, Zhou X, Du X. Circulating long non-coding RNAs in cancer: Current status and future perspectives. Molecular Cancer. 2016;**15**. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4869386/

[118] Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, et al. DD3: A new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Research. 1999;**59**(23):5975-5979

[119] de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Research. 2002;**62**(9):2695-2698

[120] Fradet Y, Saad F, Aprikian A, Dessureault J, Elhilali M, Trudel C, et al. uPM3, a new molecular urine test for the detection of prostate cancer. Urology. 2004;**64**(2):311-315; discussion 315-316

[121] Tinzl M, Marberger M, Horvath S, Chypre C. DD3PCA3 RNA analysis in urine--a new perspective for detecting prostate cancer. European Urology. 2004;**46**(2):182-186; discussion 187

[122] Wang X-S, Zhang Z, Wang H-C, Cai J-L, Xu Q-W, Li M-Q, et al. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2006;**12**(16):4851-4858

[123] Isin M, Ozgur E, Cetin G, Erten N, Aktan M, Gezer U, et al. Investigation of circulating lncRNAs in B-cell neoplasms. Clinica Chimica Acta. 2014;**431**:255-259