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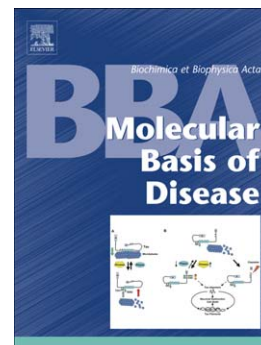
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miR-155 gene: a typical multifunctional microRNA

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Abbreviations: miR, microRNA gene; miRNA, microRNA; pri-miRNA, primary transcripts microRNA; DGCR8, Digeorge syndrome critical region gene 8; TRBP, TAR RNA binding protein; PACT, PKR-activating protein; RISC, RNA induced silencing complex; AGO, Argonaute; siRNAs, small interfering RNAs; CLL, Chronic Lymphocytic Leukemia; PD, Parkinson's disease; AD, Alzheimer's disease; FGF20, fibroblast growth factor 20; BACE 1, β -amyloid precursor protein-cleaving enzyme 1; AT1R, type I Angiotensin II receptor; BIC, B-cell Integration Cluster; HSC, hematopoietic stem cell; LPS, lipopolysaccharide; poly IC, polyriboinosinic-polyribocytidylic acid; TNF- α , Tumor Necrosis Factor- α ; JNK, c-Jun N-terminal kinase; RA, Rheumatoid Arthritis; BCR, B cell receptor; TCR, T cell receptor; MMPs, matrix metalloproteinase; AID, activation-induced cytidine deaminase; FADD, Fas (TNFRSF6)-associated via death domain protein; IKK ϵ , I κ B kinase epsilon; RIPK1, Receptor (TNFRSF)-interacting serine-threonine kinase 1; TP53INP1, tumor protein p53 induced nuclear protein 1; PMBL, Primary Mediastinal B-cell Lymphoma; DLBCL, Diffuse Large B cell Lymphoma; GCB, germinal center B cell-like; ABC, activated B cell-like; ALL, Acute Lymphoblastic Leukemia; AML, Acute Myeloid Leukemia; PDAC, Pancreatic Ductal Adenocarcinoma; Ang II, angiotensin II; KSHV, Kaposi's-sarcoma-associated herpes virus; EBV, Epstein-Barr virus.

Summary

In the last years small RNA molecules, i.e. microRNA (miRNA) encoded by miR genes, have been found to play a crucial role in regulating gene expression of a considerable part of plant's and animal's genome. Here, we report the essential information on biogenesis of miRNAs and recent evidence on their important role in human diseases. Emphasis has been given to miR-155, since this molecule represents a typical multifunctional miRNA. Recent data indicate that miR-155 has distinct expression profiles and plays a crucial role in various physiological and pathological processes such as haematopoietic lineage differentiation, immunity, inflammation, cancer, and cardiovascular diseases. Moreover, miR-155 has been found to be implicated in viral infections, particularly in those caused by DNA viruses.

The available experimental evidence indicating that miR-155 is overexpressed in a variety of malignant tumors allows us to include this miRNA in the list of genes of paramount importance in cancer diagnosis and prognosis. Exogenous molecular control in vivo of miR-155 expression could open up new ways to restrain malignant growth and viral infections, or to attenuate the progression of cardiovascular diseases.

1. Introduction

Micro-RNAs (miRNAs) are a class of evolutionarily conserved, single-stranded non-coding RNA molecules of 19-24 nucleotides that control gene expression at a post-transcriptional level. They derive from the metabolic processing of long RNA transcripts encoded by miR genes. They have been detected in plants, animal species and viruses, and are involved in numerous cellular processes including proliferation, differentiation, apoptosis and metabolism [1]. In most cases, miRNAs are able to keep under tight control cell functions by means of their capacity of gene or gene cluster silencing. To date they have been predicted to target and control the expression of at least 30% of the entire mammalian genome [2]. Of primary medical importance are the findings that deregulated miR gene expression is involved in different human diseases including cancer [3].

1.1 Biogenesis of miRNA

In the last few years, it has been found that miR genes may be present either as single units scattered in the genome or may be organized in gene clusters. A typical example of these gene clusters is provided by the miR-17-92 cluster, which comprises six miRs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1). Clustered miRs are likely transcribed together in polycistronic transcripts and usually control mRNAs with related functions. Therefore, genomic regions around known miR gene locations are particularly promising for finding new miRNA-coding DNA sequences [4]. Some miRs can be grouped as “families”. They have been identified studying sequence homology that is mainly present at the 5' end of mature miRNA [5].

An interesting aspect of miR genes is related to their genome position. They are mainly located within introns and intergenic regions, largely composed by non-coding DNA sequences previously considered as “junk DNA”.

Biogenesis of miRNA is a complex multi-step process starting in the nucleus and ending in the cytoplasm throughout many post-transcriptional modifications. In the first step of this process the RNA polymerase II transcribes miR genes generating primary transcripts (pri-miRNA) with a 5'-cap and a 3' poly-A tail. Pri-miRNAs are large transcripts that can contain multiple miRNA sequences which at this stage are already folded into hairpin structures (Fig.1). The nuclear microprocessor that contains the RNase III enzyme *Drosha* and a double-stranded-RNA-binding protein DGCR8 (*Digeorge syndrome critical region gene 8*) processes the pri-miRNA into ~70 nucleotide miRNA

precursor known as pre-miRNA. The pre-miRNA stem-loop structures are then transported to the cytoplasm through the *exportin 5 complex* [6]. The cytoplasmic endonuclease *Dicer*, complexed with its co-factors *TAR RNA binding protein* (TRBP) and *PKR-activating protein* (PACT), starts to process the pre-miRNA [7, 8]. *Dicer* cleaves the pre-miRNA stem-loop forming a 21-24 bp duplex miRNA, which contains 2 nucleotides that overhang at the 3' of each strand. One strand is then selected on the basis of the stability of the 5' end (guide strand) whereas the other strand (passenger strand) is usually degraded. At this stage the miRNA is loaded into the *RNA induced silencing complex* (RISC) containing *Argonaute* (AGO) proteins [9, 10]. After integration into the active RISC complex, miRNAs base pair with their complementary mRNA molecules. Degradation of the target mRNA occurs only when the miRNA and the target mRNA are exactly (perfect match) or nearly exactly complementary to each other. This process is the same of the RNA interference induced by artificial small interfering RNAs (siRNAs). On the other hand, if the complementarity between miRNA and target mRNA is only partial (imperfect match), translation repression will take place. In animals, miRNA molecules can bind several target sequences, mainly present at the 3' untranslated region (3'UTR) of mRNA with various degrees of complementarity [11]. It follows that each single miRNA is able to interact and regulate a large number of genes. It has been estimated that a single miRNA molecule is able to bind about 200 different transcripts [12]. In turn, biogenesis and functions of miRNA are finely governed by transcription factors and dynamic cellular signals [13].

1.2 Role of miRNA in diseases

In humans, more than 800 miR genes have been identified even if the total number is estimated to be not less than one thousand. Although research on miRNAs is only at the beginning, it is already clear that they are involved in a broad-spectrum of diseases, including cancer. The role of miRNAs in malignant transformation and tumour progression emerged with the finding that miR-15a and miR-16 are located on chromosome 13q14.3 within a 30-kb region that is frequently deleted in Chronic Lymphocytic Leukemia (CLL) determining down-regulation of both miRNAs in the majority of the analyzed CLL cell samples [14]. Interestingly, more than 50% of miR genes were found to be located at fragile sites or at chromosomal regions showing amplification, deletion or translocation in cancer [15]. Deregulated miRNA expression has now been associated with several tumour types including colon, breast, and lung cancer [16,17]. Moreover, altered miRNA expression has been found in

haematological malignancies, including several types of lymphoma and leukemia [15,18,19] suggesting that miR genes play a key role as oncogenic or tumour suppressor genes.

A certain number of miRNAs has also been found to be implicated in neurological diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD). In PD, *fibroblast growth factor 20* (FGF20) was previously identified as a risk factor in several association studies. A risk allele stems from a single nucleotide polymorphism in the 3'UTR of FGF20, responsible of disruption of a binding site for microRNA-433. This is followed by increased translation of FGF20 leading to α -synuclein over expression, which has previously been shown to cause PD.

Little is known about the role of miRNAs in AD. A number of experimental and clinical data suggest that β -amyloid is central to the pathophysiology of AD and that the *β -amyloid precursor protein-cleaving enzyme 1* (BACE1) is essential for the generation of the neurotoxic β -amyloid peptide. It has been shown that miR-107 levels decrease significantly in the majority of patients including those at the early stage of the disease. In a parallel way, the BACE1 mRNA levels tend to increase as miR-107 levels decrease in the progression of AD. The 3'UTR of BACE1 mRNA can be targeted in multiple sites by miR-107 suggesting that miR-107 is one of the multifactorial causes involved in AD progression [21].

2. Expression and function of miRNA-155

The present minireview focuses on the miR-155 (Fig.2) since this molecule represents a typical multifunctional miRNA. To date, increased evidence points out that miR-155 is involved in numerous biological processes including haematopoiesis, inflammation and immunity. It is also implicated in the regulation of type 1 Angiotensin II receptor (AT1R) an important molecule that takes part in renal function and contraction of vascular smooth muscle.

Deregulation of miR-155 has been found to be associated with different kinds of cancer, cardiovascular diseases and viral infections. Since investigation on the functional activity of miR-155 started few years ago, it is reasonable to predict that many other functions will be uncovered in the near future.

MiR-155 maps within and is processed from an exon of a non-coding RNA transcribed from the *B-cell Integration Cluster* (BIC) located on chromosome 21 [22]. BIC shows strong sequence homology

among human, mouse and chicken and is highly, although not exclusively, expressed in lymphoid organs implying an evolutionary conserved function.

2.1 Hematopoiesis

More than one miRNA is involved in regulation and differentiation of cells of haematopoietic origin. MiR-155 is expressed in both lymphoid and myeloid cells but at different levels depending on the cell type. Masaki S. and colleagues have shown that during erythroid differentiation in vitro, purified human progenitors cells show a progressive decrease of miR-155 expression, that was found to be 200 fold lower in mature red cells. On the contrary, in the same cells during the last 12 days of differentiation miR-451 is up-regulated to 270-fold, thus identifying this two miRNAs as key molecules in erythroid maturation [23].

Georgantas RW III et al. found that a substantial amount of genes specifying hematopoietic cell differentiation are expressed by CD34+ hematopoietic stem cells (HSC) but are held in check by a number of miRNAs until differentiation occurs. More exhaustive information was obtained for miR-155. Chemically-induced erythroid and megakaryocytic differentiation of K562 cell line was found to be significantly reduced by target cell transduction with miR-155. The inhibitory function of this miR gene was confirmed in in vitro cultured normal HSCs in which transduction with miR-155 generated 5-fold less myeloid and 3-fold less erythroid colonies. Furthermore, in this model, colonies were much smaller than controls [24]. These results strongly suggest that miR-155 is an essential molecule in the control of human protein translation in both myelopoiesis and erythropoiesis.

2.2 Inflammation

The inflammatory response to infections involves the induction of several hundred genes, a process that must be finely regulated to achieve pathogen clearance and to prevent the pathological consequences of a deregulated gene expression.

The possibility that environmental factors, such as inflammatory ligands, may alter miR gene expression is just beginning to be explored. Recently, miR-155 has been identified and characterized as a component of the primary macrophage response to different types of inflammatory mediators. For instance, it has been shown that miR-155 is induced by bacterial lipopolysaccharide (LPS) in a human monocytic cell line [25]. Inflammatory mediators including IFN- β , *polyribinosinic-*

polyribocytidylic acid (poly IC) or *Tumour Necrosis Factor- α* (TNF- α) can induce miR-155 in macrophages and monocytes. Furthermore, pharmacological inhibition of the *c-Jun N-terminal kinase* (JNK) blocks induction of miR-155, suggesting that miR-155-inducing signals use the JNK pathway [26]. Moreover, it has been hypothesized that the regulation of miR-155 levels could be controlled, at least in part, by the mitogen-activated protein kinase (MAPK) pathway [26].

Rheumatoid Arthritis (RA) is a chronic, systemic autoimmune disorder characterized by activation of the immune system at joint level, where it leads to inflammation and irreversible damage. Stanczyk et al. found expression of miR-155 and miR-146a in synovial fibroblasts and tissue of RA patients higher than that detectable in patients with osteoarthritis. They observed a further up-regulation of miR-155 after stimulation in vitro with TNF- α , IL-1 β and toll-like receptor ligand (LPS, poly IC). Moreover, in RA synovial fibroblasts these authors found that over expression of miR-155 induced by LPS, IL-1 β and TNF- α , down-regulates production of *matrix metalloproteinase* (MMPs) 1 and 3, that are important markers of the destructive and inflammatory effects of RA. Interestingly, the expression of miR-155 in CD14+ monocytes/macrophages present in the synovial fluid of RA patients was found 4.4 fold higher than that detectable in peripheral blood CD14+ cells. In summary, miR-155 might function as a protective miRNA that locally down-regulates the expression of certain MMPs, thus controlling tissue damage due to inflammation [27].

2.3 Immunity

BIC/miR-155 expression is greatly increased in activated B and T cells, as well as in activated macrophages and dendritic cells. On the other hand, miR-155 was found to be required for lymphocyte development and generation of B and T cell responses in vivo after BCR or TCR activation. In fact, BIC/miR155 deficient mice immunized with the tetanus toxin fragment C protein are characterized by a reduced production of IgM and switched antigen-specific antibodies. Moreover, splenocytes of immunized mice failed to produce significant levels of IL-2 and IFN- γ , thus indicating an important role for miR-155 during both T-cell independent and dependent responses. In addition to defective B and T cell immunity, miR-155 deficient mice have shown an impaired antigen-presenting function of dendritic cells [28].

Comparable results were obtained by Thai et al [29] using a similar experimental mouse model. They showed that miR-155 deficient mice had reduced germinal center function, T-cell dependent antibody

response and cytokine production. The authors stressed the finding that immune responses in miR-155-deficient mice is deranged toward a Th2 pattern, with substantial increase of IL-10, a cytokine endowed with immunodepressive activity against cell-mediated responses.

The intrinsic requirement for miR-155 was studied in detail in antigen B cell responses. In miR-155-deficient mice, B cells generate reduced extrafollicular and germinal center responses and fail to produce high-affinity IgG1 antibodies [30]. Microarray analysis of wild-type and miR-155-deficient B cells activated under conditions that promote class switching to IgG1, reveals that miR-155 regulates the expression of a substantial number of target genes, many of which can be predicted by in silico analysis. One of the genes highly expressed in miR-155-deficient B cells encodes for the transcription factor PU.1 which has a highly conserved miR-155 binding site in its 3'UTR. PU.1 is known to be important in many aspects of hematopoiesis, in particular for the early activation of B cells and regulation of B cell function. Moreover, PU.1 over expression in wild-type B cells results in reduced numbers of IgG1-switched cells. These results strongly suggest that miR-155 is required for inducing down-regulation of PU.1 in antigen driven maturation and IgG1 class-switched differentiation in B cells [30].

Recently, two independent groups identified the enzyme *activation-induced cytidine deaminase* (AID) as another important target of miR-155 in vivo. AID deaminates cytosine residues and introduces U:G mismatches in DNA. Therefore the enzyme promotes high frequency mutations that are physiologically involved in somatic hypermutation required for high-affinity IgG antibody repertoire in antigen-activated B cells. Solid experimental evidence indicates that miRNA-155 is a negative regulator of AID [31, 32]. Nucleotide substitutions in the miR-155 binding site located in the 3'UTR of AID gene prolongs the half-life of its transcript and increases the amount of AID protein. Therefore, one would expect that B cells of miR-155 deficient mice would be excellent producers of high affinity IgG antibodies. However, as previously mentioned, in vivo mouse studies provided just opposite results [30], pointing out that the high-affinity antibody response is finely regulated by different gene products presumably influenced by miR-155 in different and still not entirely understood ways.

Tili et al. investigated the relationship between miR-155 and innate immunity focusing their attention on a microbial-associated activating molecule such as LPS and the pro-inflammatory cytokine TNF- α . In LPS-stimulated macrophages TNF- α is one of the main cytokines involved in the innate immunity response. Studies on mouse macrophage/monocyte leukaemia line (i.e. Raw 264.7 cells) showed

that enhancement of TNF- α by LPS is accompanied by down-regulation of miR-125b and up-regulation of miR-155. A similar expression pattern has been also found in C57BL/6 mice subjected to i.p. inoculation with LPS [33]. A number of studies suggest that miR-125b directly targets the 3'UTR of TNF- α transcripts. It follows that down-regulation of miR-125b induced by LPS removes, at least in part, the inhibitory effect of this miRNA on TNF- α translation. On the other hand, up-regulation of miR-155 provoked by exposure to LPS, enhances TNF- α production. In this case, however, the underlying mechanism has not been fully clarified. It has been hypothesized that miR-155 could increase directly TNF- α levels by augmenting transcript stability through binding to its 3'UTR. Alternatively, miR-155 could target gene transcripts coding for proteins that are known to be repressor of TNF- α translation.

The correlation between miR-155 and responses induced by LPS are consistent with the finding that miR-155 targets transcripts coding for proteins involved in LPS/TNF α signaling including the *Fas-associated death domain protein* (FADD), the *I κ B kinase epsilon* (IKK ϵ) and the *receptor (TNFR superfamily)-interacting serine-threonine kinase1* (RIPK1). Moreover, in vivo studies showed that transgenic mice over expressing miR-155 in B cell lineage (*E μ -miR-155*) produce more TNF- α when challenged with LPS and are hypersensitive to LPS/D-galactosamine induced septic shock [33] with respect to their normal counterparts. All together, these data suggest that the interplay between miR-155 and LPS/TNF- α may be implicated in the endotoxin shock.

3. miR-155 and neoplastic diseases

There are several lines of evidence that miR-155 is over expressed in a number of neoplastic diseases and that it plays a significant role in the process of carcinogenesis, acting predominantly as an oncomir [34]. Several mechanisms have been suggested to explain this biological activity. For example, miR-155 was found to be one of the most potent miRNA suppressing apoptosis in human T-cell leukemia Jurkat cells and in MDA-MB-453 breast cancer cells. The mechanism underlying the effect of miR-155 could be ascribed to a blockade of caspase-3 activity [35]. In fact, over expression of miR-155 is followed by a substantial decrease of *tumor protein 53-induced nuclear protein 1* (TP53INP1), that is a nuclear protein able to induce cell cycle arrest and apoptosis through caspase-

3 activation [36]. This role in apoptosis could be responsible for the oncogenicity of miR-155 in several types of cancer.

3.1 miR-155 and B-Cell Lymphoma

The BIC gene was originally described as a common site of viral DNA integration in virus-induced lymphomas in chicken. In humans, BIC RNA levels are low in normal lymphoid tissues but increase in various B-cell malignancies, including Hodgkin's Lymphoma and some subtypes of Non Hodgkin's Lymphoma [37, 38].

Hodgkin's Lymphoma is clinically characterized by the orderly spread of disease from one lymph node group to another and by the development of systemic symptoms in an advanced stage of the disease. High expression of BIC/miR-155 was observed in Reed-Sternberg cells of Hodgkin's Lymphoma [39] and in the lymphocytic and histiocytic cells of the variant nodular lymphocyte-predominant Hodgkin's Lymphoma [37].

The majority of Non Hodgkin's Lymphomas does not express BIC/miR-155. However, BIC was observed in Primary Mediastinal B-cell Lymphoma (PMBL), in Diffuse Large B cell Lymphoma (DLBCL) [37] and in children's Burkitt's Lymphoma [40].

PMBL is a rare form of Non Hodgkin's Lymphoma that originates from a rare type of B-cell lymphocytes in the thymus gland. Presence of BIC/miR-155 was detected in frozen tissues and PMBL cell lines [37].

DLBCL is the most common form of adult Non Hodgkin's lymphoma accounting for nearly 40% of all lymphoid tumors. DLBCL is characterized by marked clinical and pathological heterogeneity that is reflected at the molecular level. Gene expression and immuno-histochemical studies have revealed the presence of two distinct molecular subtypes of DLBCL related to the type of cell they arise from, i.e. germinal center B cell-like (GCB) and activated B cell-like (ABC). Prognosis is worse in the ABC than in GCB subtype. BIC/miR-155 is expressed at a higher level in ABC-type than in GCB-type cell lines, suggesting that the quantification of this miRNA may have a role in establishing the prognosis [18]. Recently 42 genes down-regulated in ABC subtypes were correlated with the high expression of miR-155. Although only nine of them are predicted targets of this miRNA, an indirect effect of miR-155 on the other genes cannot be excluded. Some of these genes regulated by miR-155 are implicated in immune system homeostasis and at least three of them have oncogenic effects [41].

Burkitt's Lymphoma is a very fast growing form of Non Hodgkin's Lymphoma. In tumor cells of 11 children with Burkitt's Lymphoma, Metzler M. et al. [40] found high expression of miR-155, that was more pronounced than that detectable in Acute Lymphoblastic Leukemia (ALL) of children or in mononuclear cells of healthy donors. However, these results were not confirmed by other authors [42]. To solve this controversy, different Burkitt's Lymphoma cell lines were induced to express BIC by BCR stimulation. It was shown that BIC up-regulation is not accompanied by increase of miR-155 expression suggesting two levels of regulation of mature miR-155 expression, either at transcriptional and at processing levels [43]. However, a recent study failed to show the presence of an intrinsic blockade of BIC/miR-155 processing in cultured or primary Burkitt's Lymphoma cells [44].

As previously mentioned, miR-155 targets efficiently the 3'UTR sequence of AID gene [31, 32]. This biochemical event reveals a potentially different biological role of miR-155. Although AID activity is predominantly restricted to IgG genes, off-target lesions of DNA could be responsible of double-strand breaks favouring oncogenic translocation, including Myc translocation [32]. Since miR-155 is a negative modulator of AID levels, it is reasonable to hypothesize that this miRNA could reduce the chances of malignant transformation in selected tissues that can be targeted by AID-mediated mutagenesis (32). On the other hand, a direct evidence that miR-155 is involved in B cell malignancy has been obtained experimentally in vivo in transgenic mice carrying miR-155 that is specifically over expressed in B cell lineage [45]. In this experimental model miR-155 expression is under the control of a V_H promoter and is active in the late stage of B cell development. Transgenic mice exhibit initially a preleukemic pre-B cell proliferation followed by the development of a high-grade B cell lymphoma approximately at the age of 6 months [45].

3.2 miRNA-155 and Leukemia

Quite recently, O'Connell and others, found increased expression of miR-155 in bone marrow blasts of leukaemic patients bearing M4 or M5 subtypes of Acute Myeloid Leukaemia (AML) [46]. Two experimental models were used in mouse to investigate the possible relationship between miR-155 and AML. Expanded proliferation of granulocytes/monocytes in the bone marrow was found in mice injected with HSCs transduced with miR-155. Moreover, transduction of miR-155 in cells of the mouse macrophage RAW264.7 line, induces deregulation of a large number of genes, including 89 genes that were predicted to be targets of miR-155 on the basis of in silico analysis. Ten of these

target genes (e.g. PU.1 and C/EBP β) turned out to be involved in haematopoietic and myeloproliferative disorders [46]. Modulation of PU.1 may have an oncogenic effect since it could play a role in differentiation or activation of macrophages and B-cells and in the development of myeloid leukaemia [47]. In addition, C/EBP β is a transcription factor which plays an important role during haematopoietic cell growth and differentiation and has been found to be a potential target of miR-155 in in vivo AML development [46].

Recent investigations showed that miR-155 is over expressed in CLL, [48, 49]. There are no molecular data on the role of miR-155 in CLL, although this miRNA appears to be dramatically over expressed, leading to an average up-regulation of 5.3 fold that can peak to 23 fold [48].

3.3 miR-155 and Solid tumors

miR-155 gene was found to be over expressed in several solid tumours, such as thyroid carcinoma [50], breast cancer [17, 51, 52], colon cancer [17], cervical cancer [53], pancreatic ductal adenocarcinoma (PDAC) [54, 55], and lung cancer, where it is considered to be a marker of poor prognosis [17, 56, 57]. Several studies have recently assessed the miRNA expression profiles of different types of thyroid cancer [50, 58-61]. Thyroid cancer refers to any of the four kinds of malignant tumors of the thyroid gland: papillary, follicular, medullary or anaplastic. Nikiforava et al. analyzed frozen tissues from surgically removed thyroid samples and found that miR-155 was 9.5, 5.5 and 13.2 fold over expressed in papillary, follicular and anaplastic carcinomas with respect to normal counterpart [50]. Carcinogenic mutations in thyroid cancer have been well studied. Papillary carcinomas positive for BRAF, RAS and RET/PTC mutations showed high expression levels of miR-155, revealing a significant relationship between the presence of this miRNA and those specific mutations. Moreover, the authors showed the diagnostic utility of evaluating the expression levels of some miRNAs in the preoperative assessment of thyroid nodules in samples obtained from 14 malignant thyroid tumors. A diagnostically valid assay is important to distinguish thyroid tumors from normal thyroid tissue and hyperplastic nodules. The authors analyzed the expression of miR-155 in fine-needle aspiration samples and found up regulation of this miRNA in papillary carcinoma samples compared to hyperplastic nodules [50]. Although further analyses are needed, these results suggest a diagnostically valid assay in order to distinguish thyroid tumors from normal thyroid tissue and hyperplastic nodules.

Two independent studies detected a 10-14 fold up regulation of miR-155 in PDAC with respect to normal pancreatic tissue [54, 55]. In a recent work, a loss of TP53INP1 was demonstrated in early stages of pancreatic cancer. This is a protein known to interact with transcriptional activation of p53 in response to genotoxic stress. Induction of TP53INP1 over expression in tumour cells is followed by apoptosis and cell cycle arrest. Surprisingly, in PDAC the mRNA levels of TP53INP1 are similar to those detectable in peritumoral regions, whereas the protein is almost undetectable in cancer cells. Therefore, these results point out that miR-155 operates an efficient post-transcriptional regulation of TP53INP1, as confirmed by transfection experiments. It follows that the loss of TP53INP1 in PDAC cells is probably due to miR-155 over expression [36].

4. miR-155 in Cardiovascular diseases

The renin-angiotensin system and its effector molecule *Angiotensin II* (Ang II) play an important role in the regulation of hemodynamic physiological responses such as arteriolar vasoconstriction, blood pressure, aldosterone production and renal function. Most of the physiological effects of Ang II are mediated by AT1R.

The expression of the AT1R is regulated predominantly by post-transcriptional mechanisms. Functional studies have shown that transfection of miR-155 into human primary lung fibroblasts reduces the endogenous expression of the AT1R compared with non-transfected cells. Luciferase report assays showed that miR-155 could directly bind to the 3'UTR of AT1R mRNAs and translationally represses the protein [62]. Interestingly, a single nucleotide polymorphism (+1166 A/C) in the human AT1R was known to be associated with hypertension, cardiac hypertrophy and myocardial infarction but its role remained uncertain because it is located within an untranslated region. This polymorphism was then found to overlap with the miR-155 target site in the 3'UTR of AT1R gene (Fig.2). Laboratory experiments showed that this polymorphism reduces the ability of miR-155 to interact with the *cis* regulatory site. Consequently, miR-155 cannot attenuate efficiently the translation of the receptor protein [63]. An additional hint suggesting that miR-155 could be involved in blood pressure regulation stems from the observation published by Sethupathy [64] on homozygous twins discordant for trisomy 21. These authors found that the subject with the extra chromosome over expresses miR-155 and shows impairment of AT1R accompanied by low blood

pressure. Altogether, these results seem to link miR-155 to arterial blood pressure pattern, suggesting that a deficit of miR-155 function could be implicated in hypertension and cardiovascular diseases.

5. miR-155 in Viral infection

Large experimental evidence is now available showing that miRNAs are also encoded by viral genomes. To date, known viral miRNAs derive exclusively from dsDNA viruses (herpesvirus, polyomavirus and adenovirus). Nevertheless, for other virus families, the possibility of encoding miRNAs is not excluded. Viral miRNAs have been shown to interfere with cellular mRNAs of infected cells regulating gene expression. Gottwein and colleagues showed that a miRNA encoded by Kaposi's-sarcoma-associated herpes virus (KSHV) known as miR-K12-11, functions as an orthologue of cellular miR-155 providing a replicative advantage to the KSHV through the down regulation of the expression of genes with known roles in cell growth and apoptosis [65, 66].

BIC/miR-155 expression was analyzed in a panel of Epstein-Barr virus (EBV) positive B lymphocytes expressing type III and type I latency genes and in EBV-negative cell lines. The results showed that the type III EBV-positive cells strongly express miR-155 and that the induction of miR-155 by EBV contributes to alter gene expression by targeting a number of transcriptional regulatory genes [67]. The direct targeting of the transcription factor BACH1 by miR-155 and by its viral orthologue miR-K12-11 suggests that suppression of BACH1 as well as that of other miR-155 targets could be a conserved activity of the members of herpesvirus family.

6. Conclusions

The discovery of RNA interference is one of the major scientific breakthroughs in recent years and has revolutionized the way we look at gene regulation, so that the Nobel Prize CC Mello has given his lecture with the provocative title "Return to the RNA World: rethinking gene expression and evolution" [68]. It was only in October of 2001 that three independent groups proposed the term microRNA for a distinct class of small RNAs implicated in a post-transcriptional regulatory mechanisms, recognizing that miRNA function could be more general and important than previously

thought [69-71]. After few years the number of studies on miRNAs has been exponentially increased but the scale of the phenomenon suggests that we are still at the beginning.

In this review we report the most important results referred to miR-155 function. It was shown that miR-155 has a distinctive expression pattern during the different stages of HSC maturation. Moreover, it has a key role in modulating humoral and cell-mediated immune response. Mice deficient for miR-155 are apparently normal during the first months after birth. However, later, in the course of their life, the animals develop lung and intestinal lesions compatible with a status of immunological dysfunction that was confirmed by lack of adequate immune response following intravenous sensitization with salmonella [28-30]. Worth of note is the finding that miR-155 is greatly expressed in activated B, T cells, macrophages and dendritic cells. In addition the miR-155 down-regulation in erythroid differentiation suggests a role of this gene in erythropoiesis.

MiR-155 has been also found to be involved in cardiovascular diseases. Since over expression of this miRNA is accompanied by impairment of AT1R activity and low blood pressure, it has been suggested that a deficit of miR-155 function could be implicated in hypertension. Moreover, miR-155 appears to be involved in biochemical mechanisms associated with viral infections. For example, induction of miR-155 by EBV contributes to alter gene expression in infected cells, in part targeting a number of transcriptional regulatory genes.

To date the world literature has revealed that among the presently known miRNAs, miR-155 is one of the miRNAs most consistently involved in neoplastic diseases. Indeed, the frequently detected up-regulation of miR-155 in malignant cells allows to consider this gene predominantly as an oncogene playing a role in the pathogenesis of many human cancers, such as malignancies of the haematopoietic system (i.e. Hodgkin's Lymphoma, some types of Non Hodgkin's Lymphoma, AML, and CLL) and solid tumors (i.e. breast, colon, cervical, pancreatic, lung and thyroid cancer). This hypothesis is reinforced experimentally by the finding that over expression of miR-155 in B cells of transgenic mice induces polyclonal preleukemic B cell proliferation followed by overt B cell lymphoma [45]. However, as reported by Dorsett et al [32], the restraint operated by miR-155 on AID expression, could also suggest a possible tumor suppression function of this microRNA in a selected cellular setting. In any case, the findings that miR-155 is involved in inflammatory processes including RA [27], appear to highlight a potential link between inflammation and cancer, where the up-regulation of miR-155 could play an important role. As a consequence, rather attractive is the hypothesis that early

resolution of chronic inflammation could be followed by significant reduction of carcinogenesis through, at least in part, a modification of miR-155 expression pattern.

Up to now, not entirely elucidated are the mechanisms underlying the oncogenic potential or the participation to pathogenesis of non-neoplastic diseases of miR-155. However, a number of genes implicated in differentiation, inflammation, apoptosis and transcriptional regulation, could be controlled in their expression by miR-155.

The total number of predicted targets of miR-155 that can be obtained from the miRBase Sequence database [<http://microrna.sanger.ac.uk>] is 991. Moreover, other methods of target prediction, that are not considered in this data base, can be also used, thus increasing the number of potential targets. Not all in silico predicted targets have been found to be responsive to the miRNA upon experimental validation [72]. Presently, only a limited number of these genes has been experimentally validated by Luciferase report assay after transfection with the relevant 3'UTR portion of mRNA, as summarized in Table 1. Several transcripts of genes coding for transcriptional regulatory proteins, protein receptors, kinases, nuclear and DNA binding proteins are the direct targets of miR-155. It follows that this gene could be now considered an important pleiotropic regulator of cell homeostasis.

7. Perspectives

Even though the mechanism that regulates miRNA function and expression has not been completely clarified, the information presently available (i.e. over expression in a wide type of tumors) allows us to recognize miR-155 as a gene of paramount clinical importance either in cancer diagnosis and therapy [56, 76]. It is reasonable to predict that evaluation of mRNA-155 in tissues or biological fluids could be utilized as a biochemical parameter for tumor detection and prognosis [18, 56, 57]. Moreover, a number of groups have shown that antisense oligonucleotides complementary to the guide strand of miRNAs named anti-miRNA oligonucleotides (AMO or antimirs), or antagomirs (when they are conjugated with cholesterol), can target specific miRNAs abolishing their function in in vitro cultured cells, or in vivo in mice and in monkeys [76-79]. Therefore, it could be suggested that an efficacious device able to down-regulate miR-155 expression in a clinical setting would provide a novel, and possibly specific way to control the growth of a wide range of solid and haematopoietic malignancies in conjunction with classical cytotoxic therapy. In addition, it was recently demonstrated

that a number of drugs of clinical relevance can modulate in vitro the miRNA expression in the treated cells thus suggesting that miRNAs can be suitable targets for therapeutic effects of anticancer agents [80, 81]. We are just at the beginning of a long and still unexplored avenue of highly promising field of molecular investigation. For example, if one considers the possible role of miR-155 in human pathology not involving malignant growth, no data are presently available to suggest whether drugs used in various areas of therapeutic intervention (e.g. immune-modulating cytokines, haematopoietic factors, anti-inflammatory and immune-active agents, antiviral agents, cardiovascular drugs and other agents acting directly or indirectly on the functional activity of vital organs) are able to influence the expression of this miR gene. Considering the multi-target influence afforded by a single miRNA, it is reasonable to hypothesize that studies directed at establishing the effect of drugs on miR gene expression (i.e. a novel “pharmacomirnomics” area of investigation) could disclose possible and up-to now unrevealed mode of action of drugs. This would appear to be particularly attractive in those cases in which the pharmacological agents are known to possess pleiotropic effects that could be explained through a miRNA-mediated mechanism.

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Figure legends

Figure 1.

Biogenesis of mature miRNA.

Transcription of a miR gene by RNA polymerase II generates pri-miRNA (with a 5' 7mG cap and a 3' poly-A tail) that is processed in the nucleus into pre-miRNA by a complex containing Drosha and DGCR8 proteins. Pre-miRNA is exported to the cytoplasm and is processed into a duplex miRNA by another complex in which Dicer, TRBP, PACT proteins play the main role. After cleavage by the Dicer complex the resulting miRNA duplex is unwound and the passenger strand is degraded. The mature miRNA is loaded into the RISC to bind the mRNA target. If the complementarity between the 3'UTR mRNA and the miRNA is extensive the target mRNA is degraded, whereas, if the complementarity is partial, the translation of the target mRNA is repressed.

Figure 2.

Maturation and interaction of miR-155 with a validated mRNA target.

A. Pre-miR-155 stem-loop structure. Nucleotide sequence of miR-155 RNA duplex is indicated in red. B. Mature single strand miR-155. C. Example of interaction of miR-155 with target 3'UTR of mRNA transcript of AT1R gene. In this case, a 50% mismatch has been found between miR-155 sequence and the major AT1R allele. The arrow indicates the base that can be substituted by C in the case of single nucleotide polymorphism that is accompanied by failure of translation control by miR-155 [64].

Table 1. Direct targets of miR-155

Gene symbol	Full gene name	References
<i>Transcriptional regulatory genes</i>		
ARID2	AT rich interactive domain 2	[67]
BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor1	[46, 67]
C/EBPβ	CCAAT/enhancer binding protein, beta	[46, 67]
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1	[73]
HIF	Hypoxia-inducible factor 1	[46, 67]
HIVEP2	Human immunodeficiency virus type 1 enhancer binding protein 2	[67]
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	[28]
MEIS1	Meis homeobox 1	[73]
PU.1 (SPI1)	Spleen focus forming virus (SFFV) proviral integration oncogene spi1	[30, 46, 67]
SMAD5	SMAD family member 5	[67]
ZIC3	Zic family member 3	[67]
ZNF652	Zinc finger protein 652	[67]
<i>Protein receptors</i>		
AT1R	Angiotensin II receptor, type 1	[63, 64]
CSF1R	Colony stimulating factor 1 receptor	[46]
<i>Kinases</i>		
IKKϵ	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase ϵ	[33]
MAP3K7IP2 (TAB2)	Mitogen-activated protein kinase kinase kinase 7 interacting protein 2	[74]
RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1	[33]
<i>Nuclear proteins</i>		
AID	Activation-induced cytidine deaminase	[31, 32]
ARNTL	Aryl hydrocarbon receptor nuclear translocator-like	[46]
CUTL1 (CUX1)	Cut-like homeobox 1	[46]
JARID2	Jumonji, AT rich interactive domain 2	[46]
TP53INP1	Tumor protein p53 induced nuclear protein 1	[36]
<i>Binding proteins</i>		
FADD	Fas (TNFRSF6)-associated via death domain protein	[33]
PICALM	Phosphatidylinositol binding clathrin assembly protein	[46]
RHOA	Ras homolog gene family, member A	[75]
SLA	Scr-like-adaptor	[46]

Figure 1

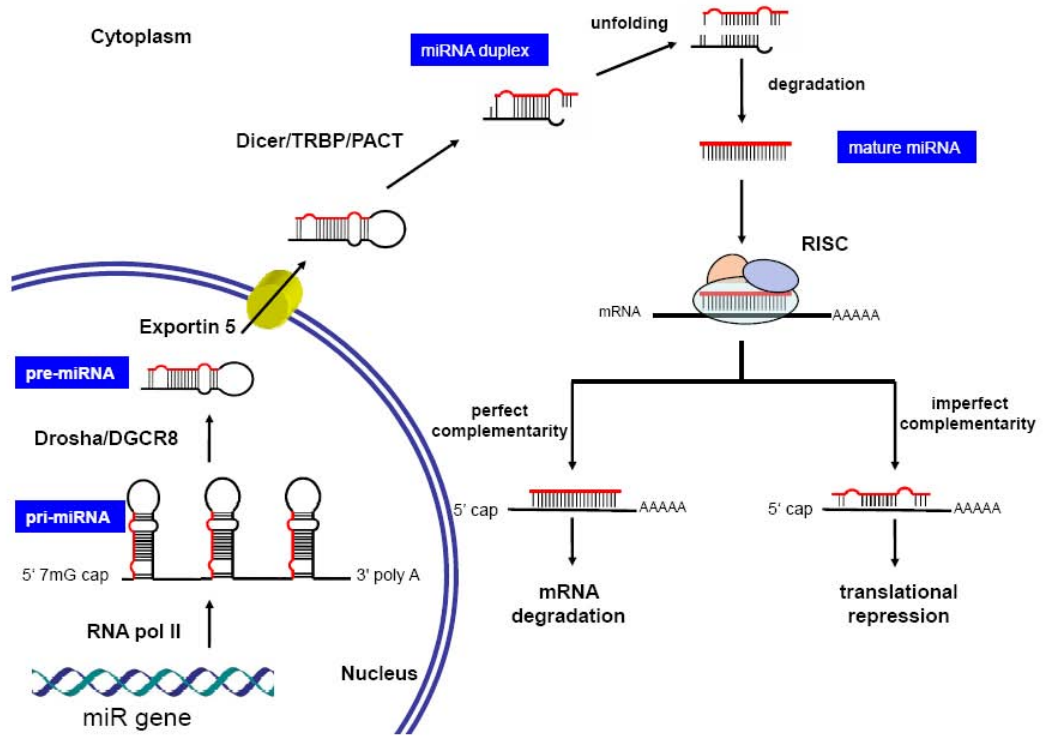
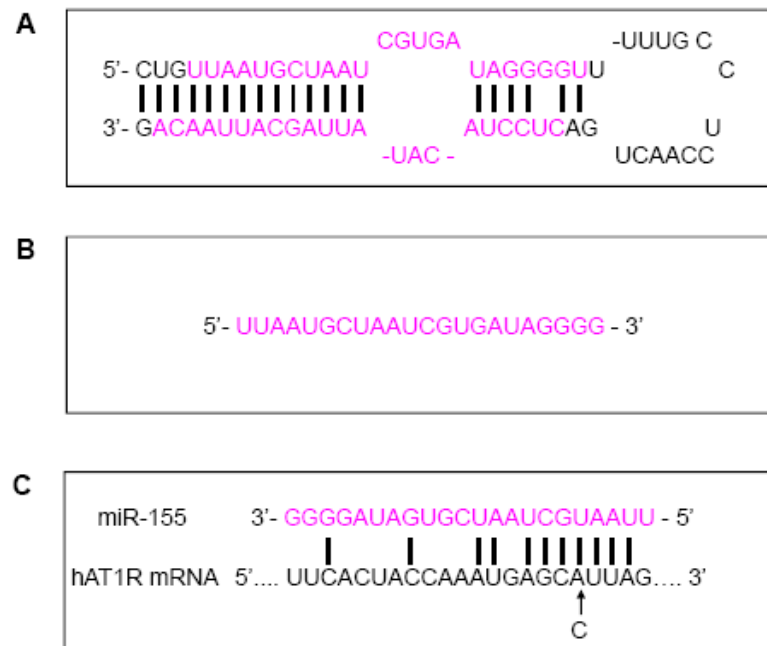


Figure 2



ACCEPTED