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A miR-155-dependent microRNA hierarchy in dendritic cell maturation and macrophage activation

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ABSTRACT

MicroRNAs (miRNAs) are small RNAs that play important regulatory roles in many cellular pathways. MiRNAs associate with members of the Argonaute protein family and bind to partially complementary sequences on mRNAs and induce translational repression or mRNA decay. Using deep sequencing and Northern blotting, we characterized miRNA expression in wild type and miR-155-deficient dendritic cells (DCs) and macrophages. Analysis of different stimuli (LPS, LDL, eLDL, oxLDL) reveals a direct influence of miR-155 on the expression levels of other miRNAs. For example, miR-455 is negatively regulated in miR-155-deficient cells possibly due to inhibition of the transcription factor C/EBPbeta by miR-155. Based on our comprehensive data sets, we propose a model of hierarchical miRNA expression dominated by miR-155 in DCs and macrophages.

1. Introduction

MiRNAs are small 21 nucleotides (nt) long RNAs with important functions in the regulation of gene expression [1,2]. MiRNAs are single stranded RNA molecules that are derived from double stranded precursor RNAs. In a step-wise process, the RNase III enzymes Drosha and Dicer cleave such miRNA precursors. Finally, the mature miRNA is incorporated into the miRNA-induced silencing complex (miRISC or miRNP) [3]. The opposing strand of the mature miRNA in the precursor molecule is termed miRNA* and this RNA is degraded during biogenesis. However, in some cases, both strands can give rise to functional miRNAs. Since miRNA* sequences can originate from either the 5′ or the 3′ arm of the precursor and it is very often not clear which strand the mature and the star sequence is, the nomenclature 'miRNA-5p' and 'miRNA-3p' is now widely used. Within miRISC, miRNAs directly bind to a member of the Argonaute (Ago) protein family and function as guides that bring Ago proteins and the miRISC to partially complementary target sites on mRNAs [4]. After the recruitment of downstream factors, the target mRNA is translationally repressed and/or degraded by exonucleases [5,6].

MiRNA-guided gene regulation has been implicated in literally all cellular pathways. In addition, most cell types including immune cells such as macrophages or DCs contain specific miRNA signatures that help to establish and maintain gene expression programs. Consequently, these miRNA signatures change during cell stimulation or differentiation and miRNAs in conjunction with transcriptional regulation lead to alterations in global gene expression. Macrophages and DCs are not only essential for the immune system but have also important functions during embryonic development and are involved in a numerous diseases including metabolic disease, atherosclerosis and cancer [7–9]. Both cell types originate from myeloid progenitor cells in the bone marrow [10]. A common progenitor that can give rise to DCs and macrophages is the monovyte. Monocytes are circulating white blood cells that can differentiate into tissue-resident macrophages or DCs in steady state and also in response to inflammation [11]. Among other white blood cells, macrophages and DCs perform phagocytosis and remove necrotic or apoptotic cells, pathogens or cell debris. In addition, macrophages contain specific receptors allowing for the uptake and clearance of different forms of low-density lipoproteins (LDL) such as oxidized LDL (oxLDL) or enzymatically hydrolyzed LDL (eLDL), which is frequently produced in plaques found in atherosclerosis [12]. Opposed to macrophages, DCs have an essential function for the initiation of adaptive immunity and upon specific stimuli, e.g. contact to a pathogen, immature DCs mature and migrate to the lymph nodes where they activate a T cell response against the pathogen. In in vitro systems, DC maturation is often achieved by lipopolysaccharide (LPS), a cell wall

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component of gram negative bacteria. DCs can also be matured by LDL. However, the physiological function of this effect remains elusive.

MiRNAs play important roles in the hematopoietic system. Many roles for miR-155 in the immune system have been reported. MiR-155 knock out studies have shown that miR-155 is important for T helper cell differentiation and thus an optimal T cell-dependent antibody response [13]. In addition, it has been demonstrated that miR-155 is also important for the function of B lymphocytes and DCs [14]. Genome-wide miR-155 target identification approaches identified regulated mRNAs important for B cell regulation, lymphocyte homeostasis as well as cytokines, chemokines and transcription factors [14,15]. MiR-155 function has also been implicated in disease such as autoimmune inflammation [16] or atherosclerosis [17]. In macrophages, miR-155 is up-regulated during inflammatory response [18] and in DCs, miR-155 together with miR-221 regulate cell development, apoptosis and interleukin-12 (IL-12) production. In addition to miR-155, many other miRNAs play important roles in macrophage and DC biology [8,19,20]. For example, miR-147 regulates inflammatory responses [21] and miR-210 regulates the production of proinflammatory cytokines in murine macrophages [22].

Although several profiling studies aiming at the identification of miRNAs that are involved in macrophage or DC functions have been performed, the interplay between individual miRNAs in such miRNA regulatory networks has not been investigated. Using cloning and deep sequencing, we have characterized miRNA expression signatures in murine bone marrow-derived macrophages and DCs stimulated or matured with LPS, LDL, oxLDL and eLDL. We performed our experiments both in wt and in miR-155 knock out cells allowing for the identification of miRNAs that are miR-155-dependent. Our approach reveals that miR-155 is a higher-ranking miRNA in macrophages and DCs that rules the expression of several lower-ranking miRNAs. This clear hierarchy leads to the establishment of miRNA signatures that are specific for individual stimuli.

2. Materials and methods

2.1. Mouse strains/preparation of primary cells

Wildtype (C57BL/6) and mir-155$^{-/-}$ [13], B6.Cg-Mir155tm1.1Rsky/J, JAX) mice used in this study were bred and maintained in a conventional animal facility according to local regulations, and sacrificed at 8–12 weeks of age for use in experiments.

DCs were generated as described previously [23]. In brief, flushed bone marrow from femur and tibia was cultured in petri dishes (Ø = 10 cm) and R10 culture medium (RPMI 1640 supplemented with 10% fetal calf serum, L-Glutamine and Penicillin/Streptomycin, all from Life Sciences) supplemented with GM-CSF hybridoma supernatant. At days 8–9 of culture, 5 × 10^5 DCs were transferred to a cell culture dish (Ø = 6 cm) and matured by overnight stimulation with either 200 ng/ml LPS (Sigma–Aldrich), 4 μg/ml LDL, oxLDL or eLDL. Macrophages were differentiated according to the DC culture but using M-CSF hybridoma supernatant instead of GM-CSF [24]. At days 8–9 of macrophage culture, confluent petri dishes were stimulated such as the DCs. For each condition, one sample was prepared for both deep sequencing and validation by Northern blotting.

2.2. Data analysis

Obtained data was analyzed using an in house written script. The data was mapped against miRBase 19 (August 2012) of mouse miRNAs (*mus musculus*). The minimal length of a read was set to 18 nucleotides, no mismatch was allowed. The reads for each miRNA were normalized against the total read number of the respective library.

For analysis of the libraries, a threshold was set to eliminate very low abundant miRNAs. For all macrophage and all dendritic cell libraries (10 libraries each), an average read number per million of total reads was calculated for all miRNAs. The cutoff was set to 5 reads per million of total reads for expression level analyses and to 50 for fold change analyses. For scatterplots with expression data, all miRNAs above threshold were plotted. For scatterplots on fold changes, again, all miRNAs above the cutoff were plotted. Venn diagrams were created using a threshold of twofold up and twofold down regulation, respectively.

2.3. Northern blot

Northern Blots were performed as described previously [25]. In short, 5–10 μg of total RNA were separated on a 12% urea gel (National Diagnostics). Ribooligonucleotides with a length of 19, 21 and 24 nt, that were labeled with γ-^{32}P-ATP prior to loading, served as a size marker. After an ethidium bromide stain, the RNA was blotted for 30 min at 20 V onto an Amersham Hybond-N membrane (GE Healthcare). The RNA was crosslinked for 1 h to the membrane using an EDC-solution [26] at 50 °C. The membrane was incubated with hybridization solution, a probe antisense to the miRNA was added and it was incubated over night at 50 °C. After several washing steps, the membrane was exposed to a screen and scanned with the PMI (Biorad).

2.4. Analysis of Col27a1 promoter region

Analysis of transcription factor binding sites was performed using AliBaba2.1. 5 kb upstream of the transcription start site of *Col27a1* (NM_025685.3) and the intron sequence upstream of *miR-455* (NR_030477.1) were tested for binding sites of C/EBPbeta.

3. Results

3.1. MiRNA expression patterns in differentially matured DCs

Immature DCs mature upon contact with various stimuli. To analyze whether different stimuli cause different miRNA expression responses in monocyte-derived murine DCs, we matured DCs with LPS, oxLDL, eLDL and LDL (Fig. 1A). Small RNAs were extracted, cloned and sequenced. While many miRNAs are expressed upon DC maturation independently of the specific maturation agent several miRNAs appeared to be stimuli-specific (Fig. 1B and C, Suppl. Tables 1 and 2). For example, 14 miRNAs including miR-155 (both strands), miR-383-5p, miR-455 (both strands), miR-9-3p or miR-149-5p were up-regulated under all four conditions while miR-99b-3p, miR-143-3p, miR-181b-1-3p and let-7e-5p were down-regulated (Fig. 1B and C, shown in blue). Generally, the miRNA profiles obtained upon maturation with LDL, oxLDL or eLDL overlap stronger compared to maturation with LPS. Nevertheless, we identified also stimuli-specific miRNAs (e.g. miR-19b-3p or miR-21a-3p for oxLDL, miR-29b-3p or miR-101a-3p for eLDL and miR-15b-3p or miR-92b-3p for LDL maturation (Fig. 1B and C)). LPS-maturation revealed the largest set of specific miRNAs including miR-210-5p, miR-96-5p, which are up-regulated (Fig. 1B) and miR-33-5p, miR-148a-5p or miR-351-5p that are down-regulated (Fig. 1C). Taken together, our comprehensive miRNA sequencing approach identifies miRNA signatures that are specific to different stimuli leading to DC maturation.

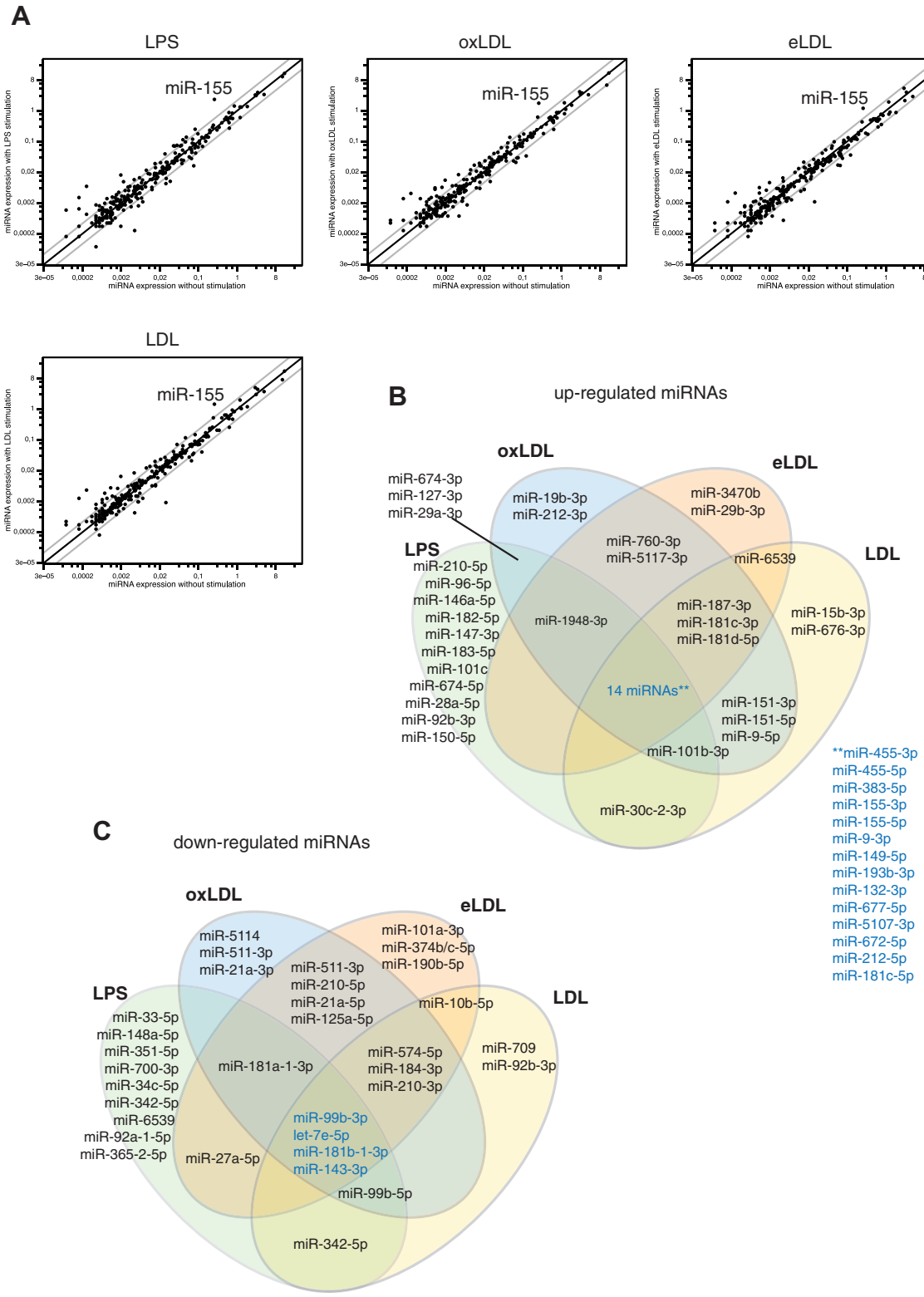

Fig. 1. (A) MiRNA expression levels of wild type (wt) immature dendritic cells (DCs) are plotted against the miRNA expression levels of matured DCs (mDCs). Stimuli used were LPS (top left), oxLDL (top middle), eLDL (top right) and LDL (bottom left). (B) Venn diagram showing up-regulated (>2-fold up) miRNAs in mDC maturation. (C) Venn diagram showing down-regulated (>2-fold down) miRNAs in mDC maturation.

3.2. MiRNA expression patterns in differentially stimulated macrophages

Not only DCs can be matured by LPS, LDL, oxLDL or eLDL but also macrophages can be stimulated with these agents. To identify miRNA regulatory networks in macrophages, we analyzed miRNA expression in differentially stimulated murine bone marrow-derived macrophages (Fig. 2). Macrophages were treated with LPS, oxLDL, eLDL and LDL, small RNAs were isolated, cloned and analyzed by deep sequencing (Suppl. Table 1). Similar to DCs, several

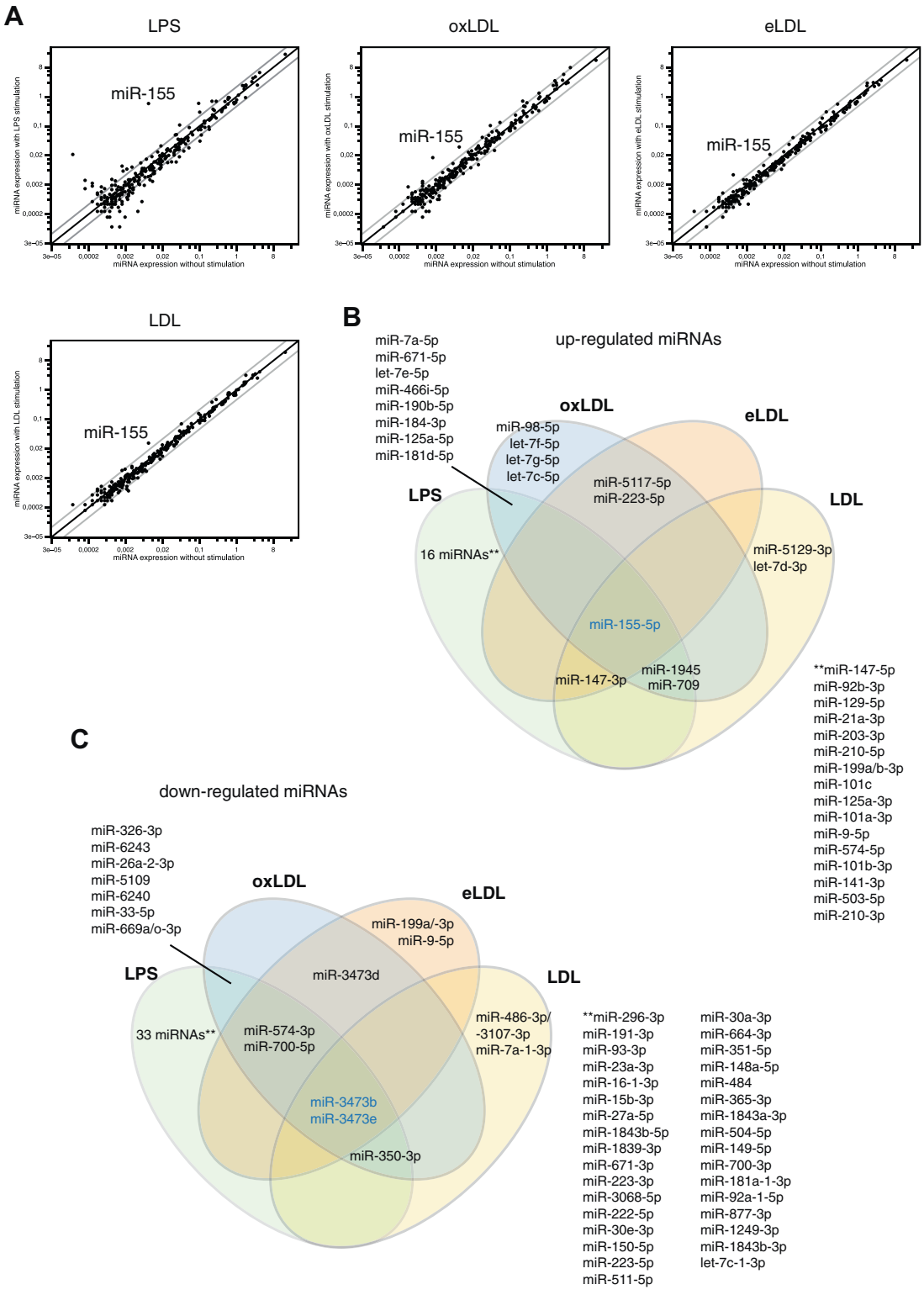

Fig. 2. (A) MiRNA expression levels of wild type (wt) unstimulated macrophages are plotted against the miRNA expression levels of stimulated macrophages. Stimuli used were LPS (top left), oxLDL (top middle), eLDL (top right) and LDL (bottom left). (B) Venn diagram showing up-regulated (>2-fold up) miRNAs in stimulated macrophages. (C) Venn diagram showing down-regulated (>2-fold down) miRNAs in stimulated macrophages.

miRNAs were differentially expressed (Fig. 2A). Interestingly, only miR-155 was up-regulated and miR-3473b/e was down-regulated under all four conditions (Fig. 2B and C, shown in blue). We also identified miRNAs that are specifically regulated by the individual stimuli in macrophages. For example, let-7f, g and c (all 5p strand) as well as miR-98-5p are up-regulated upon oxLDL stimulation, let-7a and e (both-5p) are also up-regulated as observed for LPS stimulation. Again, LPS stimulation had the strongest effect on miRNA expression: miR-147-5p, miR-92b-3p, miR-129-5p or miR-21a-3p were up- und miR-93-3p, miR-191-3p or

Table 1
miRNAs commonly regulated in macrophages and DCs.

LPS (up)	LPS (down)	oxLDL (up)	oxLDL (down)	eLDL (up)	eLDL (down)	LDL (up)	LDL (down)
miR-155-5p	miR-33-5p	miR-155-5p	no overlap	miR-155-5p	no overlap	miR-155-5p	no overlap
miR-210-5p	miR-27a-5p	miR-5117-5p		miR-5117-5p			
miR-147-3p	miR-148a-5p	miR-181d-5p					
miR-101c	miR-351-5p						
miR-101b-3p	miR-700-3p						
miR-92b-3p	miR-223-5p						
	miR-181a-1-3p						
	miR-92a-1-5p						

miR-27a-5p were down-regulated. Similar to DCs, our comprehensive sequencing analysis uncovered a number of stimuli-specific miRNAs upon macrophage stimulation. Finally, we compared stimuli-specific miRNA regulation between DCs and macrophages to identify miRNAs that are generally affected by the used stimuli. Indeed, several miRNAs overlapped between DCs and macrophages upon LPS, oxLDL and eLDL treatment (e.g. miR-155-5p, miR-210-5p, miR-147-3p, miR-33-5p or miR-27a-5p; Table 1).

3.3. Identification of miR-155-dependent miRNAs in DCs and macrophages by comparison of fold changes

MiR-155 is one of the most important miRNAs in the hematopoietic system. We hypothesized that miR-155 not only affects the expression of mRNAs but maybe also of other miRNAs. In order to identify DC miRNAs that are dependent on miR-155 and are regulated upon maturation, small RNAs were cloned and sequenced from bone marrow-derived DCs originating from wt or miR-155

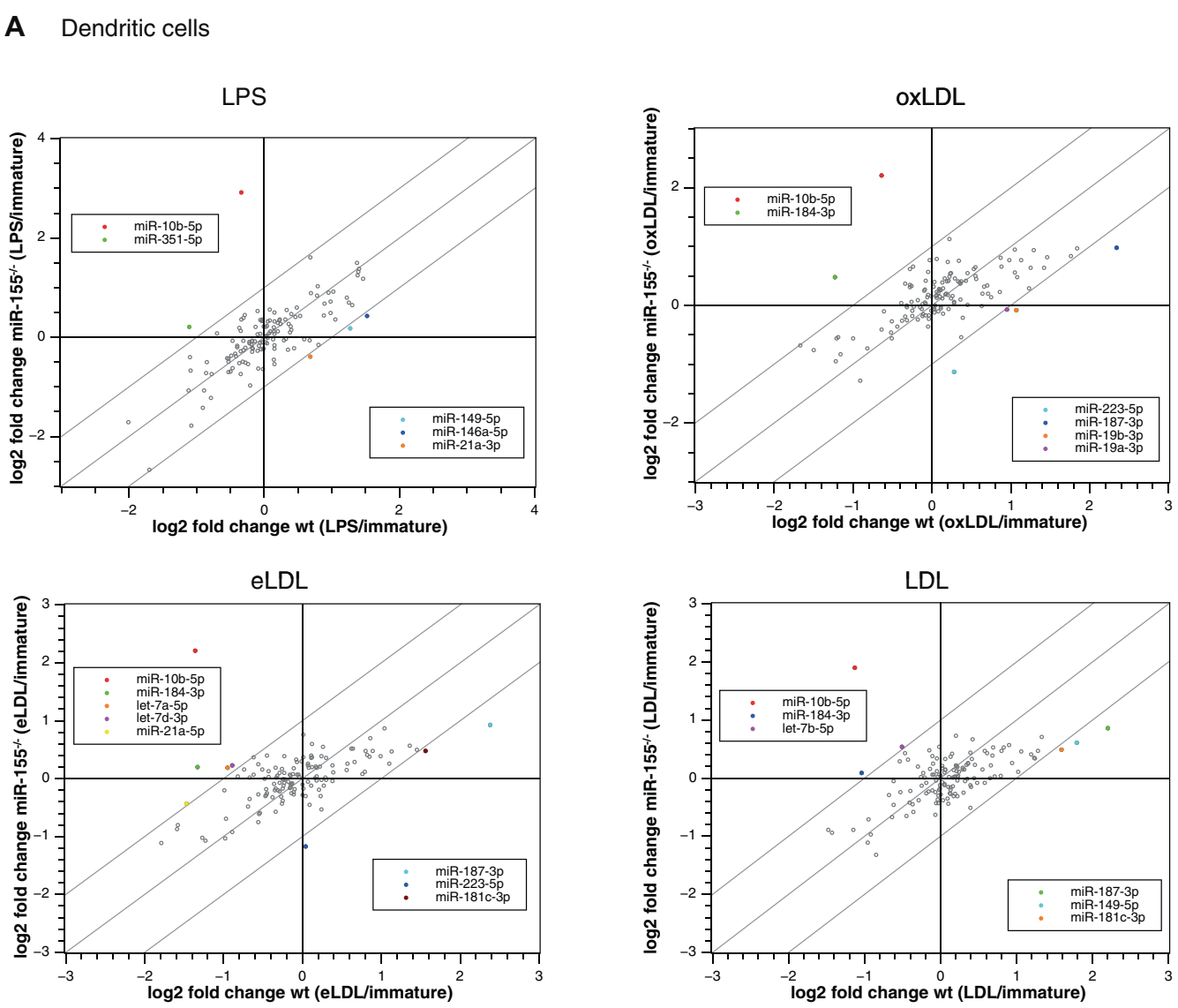

Fig. 3. (A) Scatter plots comparing fold changes in the wild type and the miR-155 deficient condition in DCs. Outer lines mark a difference of 2-fold mis-regulation. (B) Scatter plots comparing fold changes in the wild type and the miR-155 deficient condition in macrophages. Outer lines mark a difference of 2-fold mis-regulation.

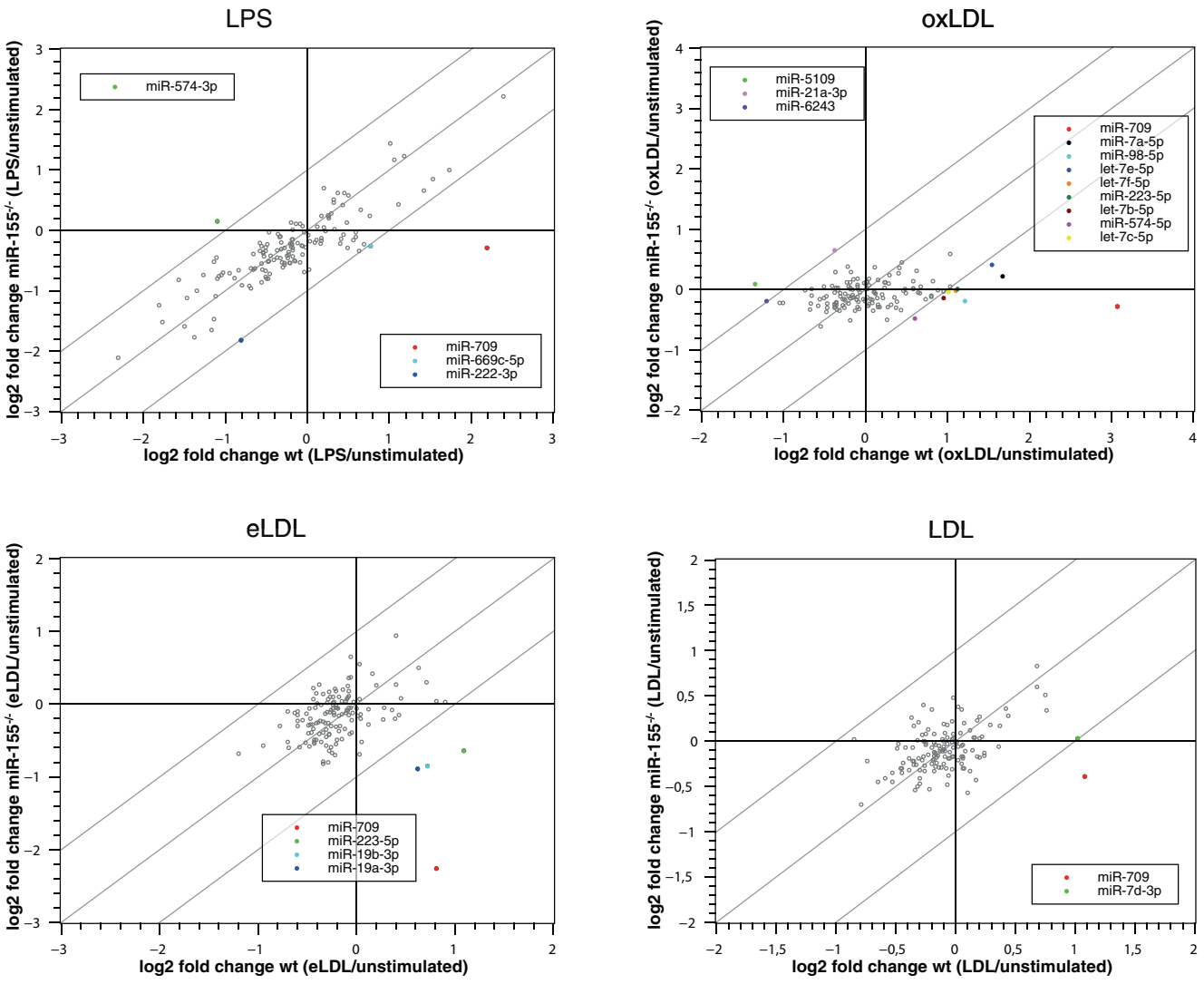

Fig. 3 (*continued*)

deficient mice (Fig. 3A, Suppl. Table 1). Wt cells and miR-155$^{-/-}$ cells were analyzed separately and their fold changes between immature cells and cells treated with the different stimuli were plotted against each other to find miR-155-dependent miRNAs (Fig. 3). A threshold of 50 reads per million of total reads was chosen to reduce low abundant and probably biologically irrelevant miRNAs. In such plots, miR-155-independent miRNAs are found between the two grey lines (indicating a 2-fold change), while miR-155-dependent miRNAs are found as outliers (colored dots). Several miRNAs were up- or down regulated in a miR-155-dependent manner in LPS (Fig. 3A, upper left), oxLDL (upper right), eLDL (lower left) or LDL (lower right) matured DCs. For example, miR-10b-5p was mis-regulated in miR-155$^{-/-}$ cells in all four different stimuli. MiRNAs miR-184-3p and miR-187-3p were affected in three out of four conditions.

We next analyzed miR-155-dependent miRNAs during macrophage stimulation (Fig. 3B). To identify miR-155-dependent miRNAs, we stimulated wt and miR-155$^{-/-}$ macrophages with LPS, LDL, oxLDL and eLDL, isolated small RNAs and analyzed them by deep sequencing. Similar to DCs, we plotted the fold changes (stimulated vs. unstimulated) of all miRNAs found in miR-155$^{-/-}$ cells against the fold changes of miRNAs found in wt macrophages (Fig. 3B). Again, we found several miRNAs whose expression depends on miR-155. MiR-709 was affected under all four conditions and miR-223-5p was mis-regulated in two out of four conditions. Again our data indicate that a miR-155-dependent miRNA network exists in macrophages as well. Our sequencing data suggests that several miRNAs might be affected by miR-155 during the maturation of bone marrow-derived murine DCs and macrophages suggesting a hierarchical system directed by miR-155.

3.4. Identification of miR-155-dependent miRNAs in DCs and macrophages by absolute expression levels

Comparing fold changes with each other might lead to a loss of miRNAs that do not pass our filters but could nevertheless be significantly up or down regulated based on absolute expression levels. Therefore, we compared expression levels between wt and miR-155$^{-/-}$ DCs (Fig. 4A, left) and macrophages (Fig. 4A, right) with each other. Indeed, we identified several miRNAs that are

differentially expressed, among them are miR-455-3p, miR-143-3p and miR-210-3p.

To consolidate our model of miR-155-dependent miRNA expression, we validated the expression levels of several miRNAs in DCs by Northern blotting (Fig. 4B). Analysis of miR-155 confirmed that the miR-155$^{-/-}$ DCs are indeed miR-155 deficient. MiR-210-3p is down regulated upon all four stimuli in wt cells. However, in miR-155 deficient cells, miR-210-3p expression is significantly reduced indicating that miR-155 influences miR-210-3p expression in DCs. Similar results were obtained for miR-143-3p. Furthermore, miR-455-3p is expressed at rather low levels in immature wt and miR-155 deficient DCs. Upon stimulation, miR-455-3p is much stronger expressed when miR-155 is not present indicating that miR-155 inhibits the expression of miR-455-3p under these conditions. In contrast, miR-181a-5p is rather independent of miR-155 expression.

We next validated our macrophage sequencing results by Northern blotting (Fig. 4C). While miR-155, miR-147-3p and miR-143-3p were not found in miR-155-deficient macrophages, miR-99b-5p was slightly down-regulated and miR-210-3p rather unaffected. Therefore, we clearly show that several miRNAs are affected by the expression of miR-155 in macrophages as well. These

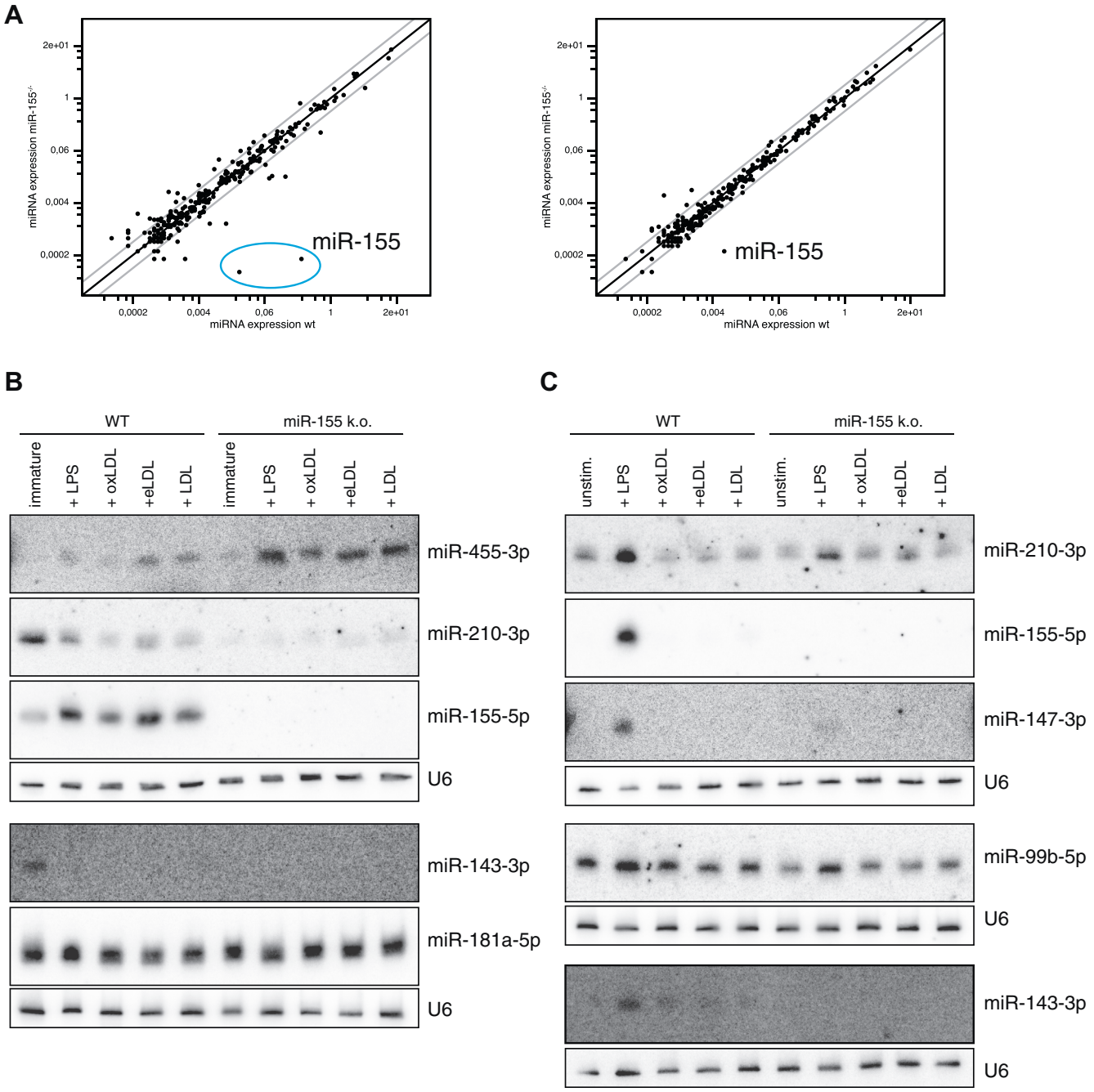

Fig. 4. (A) Scatter plots comparing miRNA expression in wt versus miR-155 deficient immature DCs (left panel) and unstimulated macrophages (right panel). Outer lines mark a 2-fold up- or down-regulation. The blue circles highlight miR-155 expression (both 5p and 3p for dendritic cells, 5p for the macrophages). (B) and (C) Expression profiles of individual miRNAs in DCs and macrophages, respectively, validated by Northern blotting. Wt and miR-155 deficient DCs (B) or wt or miR-155 deficient macrophages (C) were treated with LPS, oxLDL, eLDL and LDL and individual miRNAs were analyzed by Northern blotting. Northern blots using porbes specific to U6 were used as loading control for each specific membrane. The Northern Blots were performed using RNA from one experiment ($n = 1$).

results confirm a miR-155-dependent miRNA hierarchy during DC maturation and macrophage stimulation.

3.5. MiR-155 targets C/EBPbeta leading to miR-455 regulation

In order to identify the molecular mechanisms underlying miR-155-regulated miRNAs, we investigated miR-455-3p expression, which is up-regulated in miR-155 deficient cells (Fig. 4B). It has been demonstrated before that miR-155 regulates the transcription factor C/EBPbeta and affects adipocyte differentiation [27]. Therefore, we searched for putative C/EBPbeta binding sites close to potential *MiR-455* promoters (Fig. 5A). Indeed, three potential sites are found in the *MiR-455* containing intron of the *Col27a1* gene. In addition, another four binding sites are present at the *Col27a1* promoter. Since miR-155 is induced the strongest in cells stimulated with LPS, we next analyzed C/EBPbeta levels after LPS maturation in wt and miR-155$^{-/-}$ DCs (Fig. 5B). Upon LPS treatment, C/EBPbeta is down-regulated, which is consistent with the observed miR-155 increase. Strikingly, C/EBPbeta is strongly elevated in miR-155$^{-/-}$ cells matured with LPS, which in turn might lead to up regulation of miR-455-3p (Fig. 5B and C).

4. Discussion

MiRNAs are regulatory molecules that help to establish gene expression profiles by regulating protein-coding genes. Such regulatory networks lead to the manifestation of cell identity. Thus, miRNA expression profiles are specific to cell types and also the target repertoire is often cell type-specific. Although very often not well understood in molecular detail, the general effects of miRNAs on mRNA target expression are rather clear. In contrast, consequences of miRNAs on the expression of other miRNAs have not been analyzed. Here, we have characterized miRNA expression profiles in wt DCs or macrophages and compared them with profiles from miR-155$^{-/-}$ DCs or macrophages. This approaches allows for the establishment of a miRNA hierarchy directed by miR-155. In macrophages, the expression of miR-99b-5p, miR-147-3p and miR-143-3p is stimulated by miR-155, while miR-709 and

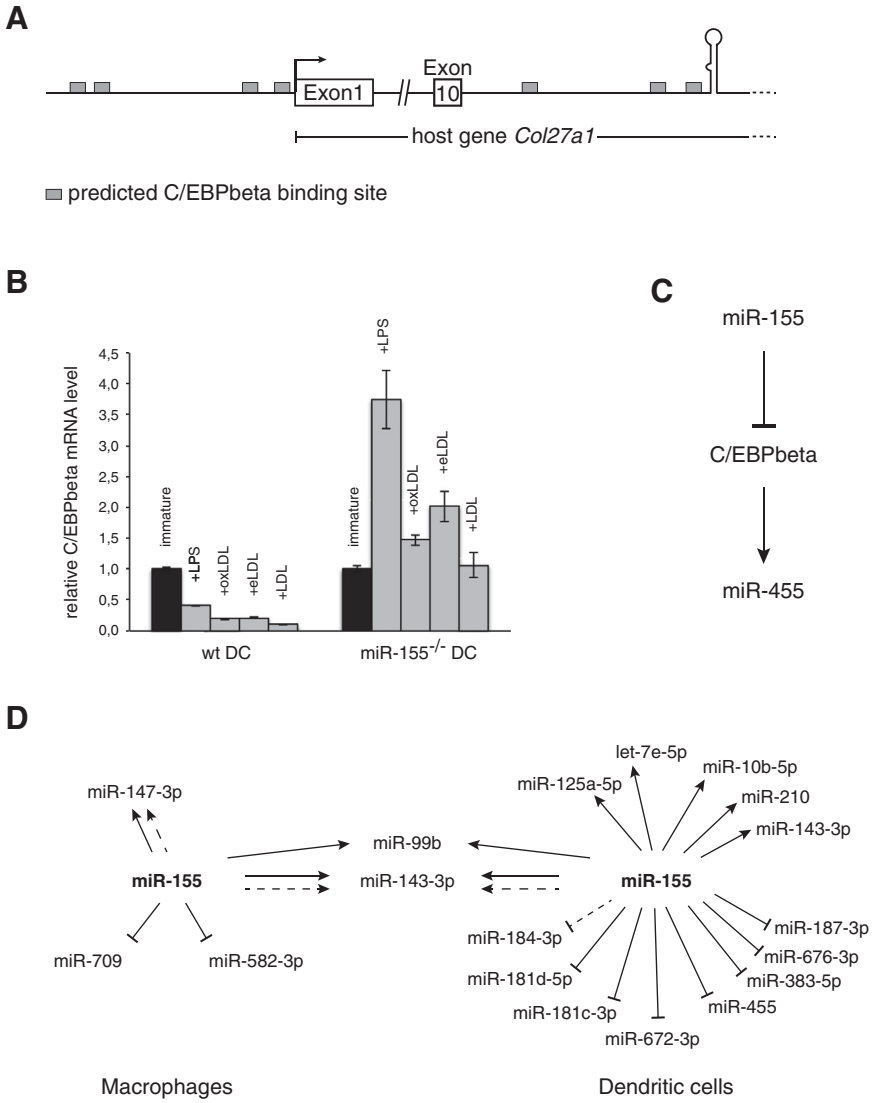

Fig. 5. (A) Schematic representation of the promoter region of the putative host gene of miR-455, *Col27a1*, and the upstream intronic sequence of miR-455. Predicted binding sites for the transcription factor C/EBPbeta are shown as blue boxes. (B) qPCR measurement of *C/EBPbeta* mRNA in immature and matured (LPS, oxLDL, eLDL, LDL) dendritic cells, both in wild type and in miR-155 deficient cells. Levels were normalized to the *36B4* housekeeping gene and the respective level in immature cells. Dark grey, immature condition; light grey, matured cells. (C) Proposed regulatory model of miR-155 influenced miR-455 expression. (D) Summary of miRNAs regulated by miR-155. Solid arrows represent supportive roles of miR-155 in expression levels, while dotted arrows represent supportive roles in expression changes (data shown in Fig. 3). Lines with a bar represent the inhibition by miR-155 in either expression level (solid line) or expression change (dotted line). Left, regulated miRNAs in macrophages, right, regulated miRNAs in DCs.

miR-582-3p are repressed (Fig. 5D). In DCs, miR-99b (both strands), miR143-3p, miR-125a-5p, let-7e-5p, miR-10b-5p, miR-210 (both strands) and miR143-3p are stimulated and miR-187-3p, miR-676-3p, miR-383-5p, miR-455 (both strands), miR-672-3p, miR-181c-3p, miR-181d-5p or miR-184-3p are repressed (Fig. 5D).

How is such a regulation achieved? Most likely, either transcriptional activators or repressors that bind to miRNA promoters and regulate miRNA expression are directly targeted by miR-155 leading to stimulation or inhibition of expression. Supporting this model, we find that miR-455 contains several C/EBPbeta binding sites at its putative promoter regions. Interestingly, C/EBPbeta is a direct miR-155 target. However, miR-455 is mildly up regulated upon maturation of wt DCs as well suggesting that additional pathways might contribute to overall miR-455 expression levels. Other transcription factors that are miR-155 targets and could affect miRNA expression are Ship1 [28], Pu.1 [29] or Socs1 [30], for example. In addition, it is also conceivable that regulatory proteins that directly interact with miRNA processing intermediates could be targeted by miR-155. Such regulatory factors are often cell-type-specific. This might explain why we observe considerable differences between miR-155-dependent miRNAs in DCs and macrophages. A better understanding of the miR-155 target spectrum will help elucidating how such a miRNA hierarchy is established and maintained.

Recently, it has been shown that miR-709 regulates miR-15a and miR-16 [31]. In this study, murine miR-709 seems to localize to the nucleus where it directly binds to the pri-miR-15a/16 transcript and inhibits its expression. Although rather low abundant, miR-709 is also among the miRNAs that are stimulated by miR-155 in our study. However, in our system, we do not observe effects on miR-15a/16 suggesting again cell-type-specific effects. Furthermore, miR-155 is not only important for macrophage or DC function but also in many other cells of the hematopoietic system such as CD8+ T cells [32] or T helper cells [13]. In addition, miR-155 is important for brown fat tissue differentiation [27] and has also been implicated in a number of different types of cancer [33]. It will be interesting to see whether miR-155 ruled miRNAs also exist in other tissues and what the consequences are for cell function and disease.

5. Description of additional data files/data deposition

Supplementary Table 1 contains all miRNA deep sequencing data obtained from DCs and macrophages. All sequencing data were deposited at GEO database ID: GSE48404.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.01.009.

References

[1] Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.
[2] Carthew, R.W. and Sontheimer, E.J. (2009) Origins and mechanisms of miRNAs and siRNAs. Cell 136, 642–655.
[3] Kim, V.N., Han, J. and Siomi, M.C. (2009) Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. 10, 126–139.
[4] Meister, G. (2013) Argonaute proteins: functional insights and emerging roles. Nat. Rev. Genet. 14, 447–459.
[5] Braun, J.E., Huntzinger, E. and Izaurralde, E. (2013) The role of GW182 proteins in miRNA-mediated gene silencing. Adv. Exp. Med. Biol. 768, 147–163.
[6] Krol, J., Loedige, I. and Filipowicz, W. (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat. Rev. Genet. 11, 597–610.
[7] Squadrito, M.L., Etzrodt, M., De Palma, M. and Pittet, M.J. (2013) MicroRNA-mediated control of macrophages and its implications for cancer. Trends Immunol. 34, 350–359.
[8] Busch, M. and Zernecke, A. (2012) MicroRNAs in the regulation of dendritic cell functions in inflammation and atherosclerosis. J. Mol. Med. (Berl) 90, 877–885.
[9] Urbich, C., Kuehbacher, A. and Dimmeler, S. (2008) Role of microRNAs in vascular diseases, inflammation, and angiogenesis. Cardiovasc. Res. 79, 581–588.
[10] Shi, C. and Pamer, E.G. (2011) Monocyte recruitment during infection and inflammation. Nat. Rev. Immunol. 11, 762–774.
[11] Murray, P.J. and Wynn, T.A. (2011) Protective and pathogenic functions of macrophage subsets. Nat. Rev. Immunol. 11, 723–737.
[12] Moore, K.J. and Tabas, I. (2011) Macrophages in the pathogenesis of atherosclerosis. Cell 145, 341–355.
[13] Thai, T.H. et al. (2007) Regulation of the germinal center response by microRNA-155. Science 316, 604–608.
[14] Rodriguez, A. et al. (2007) Requirement of bic/microRNA-155 for normal immune function. Science 316, 608–611.
[15] Loeb, G.B., Khan, A.A., Canner, D., Hiatt, J.B., Shendure, J., Darnell, R.B., Leslie, C.S. and Rudensky, A.Y. (2012) Transcriptome-wide miR-155 binding map reveals widespread noncanonical microRNA targeting. Mol. Cell 48, 760–770.
[16] O'Connell, R.M. et al. (2010) MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity 33, 607–619.
[17] Nazari-Jahantigh, M. et al. (2012) MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. J. Clin. Invest. 122, 4190–4202.
[18] O'Connell, R.M., Taganov, K.D., Boldin, M.P., Cheng, G. and Baltimore, D. (2007) MicroRNA-155 is induced during the macrophage inflammatory response. Proc. Natl. Acad. Sci. USA 104, 1604–1609.
[19] Nazari-Jahantigh, M., Wei, Y. and Schober, A. (2012) The role of microRNAs in arterial remodelling. Thromb. Haemost. 107, 611–618.
[20] Zernecke, A. (2012) MicroRNAs in the regulation of immune cell functions-implications for atherosclerotic vascular disease. Thromb. Haemost. 107, 626–633.
[21] Liu, G., Friggeri, A., Yang, Y., Park, Y.J., Tsuruta, Y. and Abraham, E. (2009) MiR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. Proc. Natl. Acad. Sci. USA 106, 15819–15824.
[22] Qi, J., Qiao, Y., Wang, P., Li, S., Zhao, W. and Gao, C. (2012) MicroRNA-210 negatively regulates LPS-induced production of proinflammatory cytokines by targeting NF-kappaB1 in murine macrophages. FEBS Lett. 586, 1201–1207.
[23] Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rossner, S., Koch, F., Romani, N. and Schuler, G. (1999) An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods 223, 77–92.
[24] Rutherford, M.S. and Schook, L.B. (1992) Differential immunocompetence of macrophages derived using macrophage or granulocyte-macrophage colony-stimulating factor. J. Leukoc. Biol. 51, 69–76.
[25] Dueck, A., Ziegler, C., Eichner, A., Berezikov, E. and Meister, G. (2012) MicroRNAs associated with the different human Argonaute proteins. Nucleic Acids Res. 40, 9850–9862.
[26] Pall, G.S. and Hamilton, A.J. (2008) Improved northern blot method for enhanced detection of small RNA. Nat. Protoc. 3, 1077–1084.
[27] Chen, Y., Siegel, F., Kipschull, S., Haas, B., Frohlich, H., Meister, G. and Pfeifer, A. (2013) MiR-155 regulates differentiation of brown and beige adipocytes via a bistable circuit. Nat. Commun. 4, 1769.
[28] O'Connell, R.M., Chaudhuri, A.A., Rao, D.S. and Baltimore, D. (2009) Inositol phosphatase SHIP1 is a primary target of miR-155. Proc. Natl. Acad. Sci. USA 106, 7113–7118.
[29] Vigorito, E. et al. (2007) MicroRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity 27, 847–859.
[30] Androulidaki, A. et al. (2009) The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. Immunity 31, 220–231.
[31] Tang, R. et al. (2012) Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: evidence for a microRNA hierarchy system. Cell Res. 22, 504–515.
[32] Dudda, J.C. et al. (2013) MicroRNA-155 is required for effector CD8+ T cell responses to virus infection and cancer. Immunity 38, 742–753.
[33] Garzon, R., Calin, G.A. and Croce, C.M. (2009) MicroRNAs in cancer. Annu. Rev. Med. 60, 167–179.