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Deletion of microRNA miR-223 increases Langerhans cell cross-presentation

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Abstract

Langerhans cells (LCs) are skin-residential dendritic cells that regulate skin immunity. MicroRNAs (miRNAs) are key regulators in the control of biological functions in a variety of cell types. Deletion of all miRNAs interrupts the homeostasis and function of epidermal LCs. However, the roles of individual miRNAs in regulating LC development and function are still completely unknown. MiRNA miR-233 is especially expressed in the myeloid compartment. Here, we reported that miR-223 is highly expressed in freshly isolated epidermal LCs, and tested whether miR-223 regulates LC development and function using miR-223 knockout (KO) mice. We found that the number, maturation, migration and phagocytic capacity of LCs were comparable between miR-223KO and wild-type mice. However, lack of miR-223 significantly increases LCs-mediated antigen-specific $CD8^+$ T cell proliferation *in vivo* and *in vitro*, while LCs from KO and WT mice showed comparable stimulation for antigen-specific $CD4^+$ T cells. Our data suggest that miR-223 negatively regulates LC cross-presentation, but may not be required for normal LC homeostasis and development.

Keywords

Langerhans cells; microRNA; miR-223; cross-presentation

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Author contributions Q.S.M and L.Z. designed experiments, Q.S.M, D.Z., and L.Z. wrote the manuscript, and all authors have read and revised the manuscript critically; R.Q., Y.P.X, H.W. and Q.S.M performed the experiments; R.Q., Y.P.X, H.W., L.Z. and Q.S.M analyzed data.

Conflicts of interest: The authors declare that there is no duality of interest associated with this study

1. Introduction

As the body's largest and most exposed interface with the environment, the skin forms a first protective barrier between the host and its external environment and is equipped with a system of immune surveillance to combat against a broad range of pathogens and control peripheral tolerance. Dendritic cells (DCs) are professional antigen presenting cells involved in initiating T cells response and peripheral tolerance. Langerhans cells (LCs) are hematopoetic stem cells–derived immature skin-residential DCs with a life cycle distinct from other types of DCs (Romani et al., 2010, Kaplan, 2010, Merad et al., 2008). With the ability to traffic peripherally acquired antigens to skin-draining lymph nodes (LNs) and present processed antigens to T cells, LCs are able to initiate adaptive immune responses as well as induce tolerance, and have been linked to the regulation of various immune responses, including allergy contact dermatitis (ACD) (Romani et al., 2010, Kaplan, 2010, Merad et al., 2008). However, the mechanisms that regulate LC homeostasis and immunological functions still remain unclear.

MicroRNAs (miRNAs) are a class of short (~22nt) noncoding RNAs that regulate gene expression through either translation repression or mRNA degradation (Lewis et al., 2003, Bartel, 2004). Accumulated evidence is emerging that miRNAs are key regulators in the control of biological processes in a variety of mammalian cell types, including immune cells (Zhou et al., 2011, Zhou et al., 2009, Zheng et al., 2012), and have been implicated in several inflammatory diseases, including atopic dermatitis and ACD (Sonkoly et al., 2010, Vennegaard et al., 2012). The ribonuclease III enzyme Dicer is required for the processing of mature and functional miRNAs. Therefore, deletion of Dicer provides a genetic test for the relevance of miRNAs in mammalian development. Using a mouse model with the deletion of Dicer, studies from our group and others indicate that lack of miRNAs by Dicer-deletion dramatically interrupts T and B cell development and function (Zhou et al., 2011, Zhou et al., 2009, Seo et al., 2010, Cobb et al., 2006, Liston et al., 2008). Interestingly, deletion of Dicer specifically in DCs only affects the homeostasis and function of LCs, but not other types of DCs, suggesting that miRNAs are required for normal LC development and function (Kuipers et al., 2010). However, the roles of specific single miRNA in this context are still lacking.

MicroRNA-223 (miR-233) was first identified bioinformatically and expressed in the hematopoietic system, especially in the myeloid compartment (Chen et al., 2004). Recent studies form Camargo's group showed that miR-223 is selectively expressed in neutrophils and macrophages. Using a miR-223 mutant mouse model, they further identified that miR-223 negatively regulates the proliferation and differentiation of neutrophils by down-regulating the transcription factor Mef2c, and miR-223 mutant mice spontaneously develop inflammatory lung pathology and exhibit exaggerated tissue destruction after endotoxin challenge (Johnnidis et al., 2008). Here, we report that miR-223 is highly expressed in freshly isolated epidermal LCs. Differentiation of LCs is absolutely dependent on TGF-β1 both *in vitro* and *in vivo* (Merad et al., 2008, Romani et al., 2010, Borkowski et al., 1996). TGF-β1 induces PU.1 expression in $CD34^+$ hematopoietic progenitor cells undergoing LC commitment, and ectopic expression of PU.1 strongly enhanced TGF-β1–dependent LC development (Heinz et al., 2006). Interestingly, the miR-223 gene is driven by PU.1, and PU.1 repression down-regulates miR-223 expression (Fukao et al., 2007). Thus, we hypothesized that miR-223 might regulate LC development and function. To test this hypothesis, we used a miR223-deficient mouse model to evaluate the role of miRNA-223 in LC development and function. Our results show that the number, maturation, migration and phagocytic capacity of LCs were comparable between miR-223KO and wild-type mice. However, lack of miR-223 significantly increases LCs-mediated antigen-specific $CD8^+$ T cell proliferation *in vivo* and *in vitro*, while LCs from KO and WT mice showed comparable

stimulation for antigen-specific $CD4^+$ T cells. Our data highly suggest that miR-223 negatively regulates LC cross-presentation, but may not be required for normal LC homeostasis and development.

2. Materials and methods

2.1. Mice

MiR-223 knockout (KO) mice were described previously (Johnnidis et al., 2008). All wild-type (WT) mice, unless indicated otherwise, are littermate controls of miR-223-KO mice. Experiments were conducted at 6–8 weeks of age, unless otherwise indicated. Mice were housed in a specific pathogen-free barrier unit. Handling of mice and experimental procedures were in accordance with requirements of the Institutional Animal Care and Use Committee.

2.2. Single cell suspension preparations and Flow Cytometry

Epidermal suspensions prepared from skin of miR-223KO and wild-type (WT) mice as described with some modifications (Kissenpfennig et al., 2005, Nagao et al., 2009) Briefly, skin was rinsed with 70% alcohol; ears were split with fine forceps and placed, dermal side down, in 0.5% dispase (Gibco) in 1×PBS (without calcium or magnesium) for 60 min at 37°C. In the case of trunk skin, the subcutaneous fat was scraped off before placing in dispase. Epidermal sheets were then peeled from the underlying dermis and floated in complete culture medium with 100μg/ml DNase (Sigma) and 0.05% Trysin in a shaking water bath for 20 min at 37°C. The single epidermal cells were harvested after filtering the cell suspension through a 70μM filter. Complete culture medium was RPMI 1640 (with 2 mM L–glutamine, Gibco) supplemented with 10% heat-inactivated FBS (Hyclone), 5×10^{-5} M 2- mercaptoethanol, 0.15% sodium hydrogencarbonate, 1 mM sodium pyruvate, nonessential amino acids, 100 U/ml penicillin, and 100μg/ml streptomycin (Gibco).

2.3. Flow Cytometry and antibodies

Epidermal cell suspensions were pretreated with 24G2 for 10 min at 4°C. Cells were then stained for extracellular markers and/or intracellular marker. The following conjugated monoclonal antibodies (mAbs) were used: I-A/E (M5/114.15.2), CD45.2, CD80 (16-10A1), CD86 (GL1), CD3e (145-2C11), CD4 (GK1.5), EpCAM (G8.8), langerin (929F3.01). All antibodies were purchased from eBiosceience or Dendritics. Cells were analyzed with BD LSR II flow cytometer or FACSAria™ II (BD Biosciences) by Flowjo.

2.4. Langerhans cell sorting

Epidermal cells were first stained with PE-labeled anti-I-A/E (M5/114.15.2) followed by anti-PE-beads, and then LCs were enriched by AutoMACS (up to 30%). The CD45.2 and MCHII double positive LCs were further sorted by a FACSAria™II Cell Sorter. The purity of Langerhans cells was up to 99%.

2.5. Culture of BMDCs from bone marrow

Bone marrow cells were flushed from femur and tibia of mice and cultured in complete medium supplemented with 10ng/ml GM-CSF. Fresh complete medium supplemented with cytokines was added every 3 days. On day 6, non-adherent bone marrow-derived DC (BMDC) were sorted by CD11c using a FACSAria™ II Cell Sorter.

2.6. Real-Time RT-PCR

RNAs from sorted BMDCs, freshly Langehans cells and splenic T cells from B6 mice were purified using the Promega miRNA isolation kit according to the manufacturer's

instructions. The expression of miR-223 was examined using the Applied Biosystems TaqMan MicroRNA Assay kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions; snoRAN135 was used as endogenous control. PCR amplification was carried out on the Applied Biosystems 7900 Real-Time PCR system.

2.7. In vitro maturation

Freshly separate epidermal cells were cultured with complete medium at 37°C for 48hrs. The cells were then collected and stained with anti-I-A/E, langerin (929F3.01), CD45.2, CD80 and CD86 for flow cytometry analyses.

2.8. Phagocytosis assay

Epidermal cells were cultured with 0.25mg/ml Dextran-Fluorescence (Invitrogen) for 45 min at 37°C or 4°C. After staining with APC-labeled anti-CD45.2 and PE-labeled anti-I-A/E, the percentage of LCs that uptake antigen ($CD45.2^+$/I-A/E /$FITC^+$) were analyzed by flow cytometry.

2.9. Isolation of T cells and in vitro proliferation assays

Spleen $CD8^+$ or $CD4^+$ T cells were sorted (from OT-I or OT-II mice, respectively) using a FACSAria™II Cell Sorter. The T cell suspensions (2.5×10^4 cells/well) were then cultured with the different ratios of LCs for 72 hrs, with proliferation measured by incorporation of [^{3}H] thymidine during the last 16 h.

2.10. In vivo OVA presentation

miR-223KO and WT mice were reconstituted with CFSE-labeled OT-I CD8 T cells. After 48h, 50μl of PBS with or without 25μg of OVA were placed on the shaved and tape-striped back skin of recipient mice. An additional 48h later, skin LNs were collected and analyzed for CFSE staining by flow cytometry to assess the specific proliferation of the adoptively transferred OT-I T cells.

2.11. Hapten sensitization and elicitation of allergy contact dermatitis (ACD)

Mice were sensitized by applying 25μl 0.5% DNFB (Sigma-Aldrich, St. Louis, MO, USA) (acetone:olive oil=4:1) topically on shaved abdominal skin on day 0. On day 5, sensitized mice were challenged topically with 10μl 0.2% DNFB on the left ear. An identical volume of acetone/olive oil was painted on the right ear. Ear thicknesses were measured in a blinded fashion by comparing challenged (left) and unchallenged (right) ears using a thickness gauge (Digimatic caliper, Mitutoyo, Japan) before and at 24, 48 72h, and 96h after the challenge, and ear thickness increases were calculated by subtracting pre-challenge (0 h) from post-challenge measurements (24h, 48h, 72h, and 96h).

2.12. Statistical analysis

The two-tailed Student's *t* test was used (GraphPad Software). Differences were considered statistically significant when $P < 0.05$.

3. Results and Discussion

miR-223 is highly expressed in the myeloid compartment. As showed in Fig. 1A, we confirmed that miR-223 is highly expressed in freshly isolated epidermal LCs and bone marrow-derive DCs compared to spleen T cells. We next tested whether lack of miR-223 could affect epidermal LC development and homeostasis using miR-223 knockout (KO) mice (Johnnidis et al., 2008). As shown in Fig. 1B, the percentage of epidermal LCs (Langerin$^+$CD45.2$^+$) in miR-223KO mice was comparable to that from WT mice. Thus,

miR-223 may not be required for LC development. LCs express some co-stimulatory molecules necessary for stimulation of T cells, including MHC-II, CD80, and CD86, when they are matured. To test the role of miR-223 in LC maturation, the frequencies and expression levels of LC maturation markers were evaluated after *in vitro* culture. As shown in Fig. 1C, the percentages of CD86-and CD80-positive LCs were not significantly different between miR-223KO and WT mice. Thus, lack of miR-223 did not block LC maturation *in vitro*. To evaluate the potential role of miR-223 in the antigen-uptake function of LCs, freshly isolated epidermal cells were incubated with FITC-dextran and then stained with anti-MHCII and CD45.2 antibodies. As shown in Fig. 1D, the phagocytic capacity of LCs from miR-223KO mice was not significantly changed compared to control mice, based on the frequencies of $FITC^+$LCs and their MFI expression. To further assess *in vivo* antigen-capture and the migration capability of LCs in miR-223KO mice, FITC was applied onto the skin as a tracer. The uptake and transport of FITC by LCs could be easily traced by fluorescence. Recent studies revealed the presence of at least 3 distinct skin DC subsets based on the differential expression of EpCAM and langerin, and epidermal LCs are $Langerin^+EpCAM^+$ DCs (Nagao et al., 2009). The proportion of $Langerin^+EpCAM^+$ cells on gated CD8-negative population in LNs, which were migrated epidermal LCs, were comparable between WT and miR-223KO mice (Fig. 1E). Furthermore, the frequency of FITC-positive LCs on gated $Langerin^+Epicam^+$ cells was also comparable between WT and miR-223KO mice (Fig. 1F). Thus, our data indicated that miR-223 is not critically required for LC antigen capture and migration to draining LNs.

The presentation of exogenous antigens on MHC class I molecules, known as cross-presentation, is essential for the initiation of $CD8^+$ T cell responses. DC cross-presentation is an important mechanism for generating immunity and tolerance. Recent studies indicated that LCs can cross-present soluble exogenous antigen ovalbumin (OVA) and cellular antigens from epidermis to antigen-specific T cells (Kissenpfennig et al., 2005, Stoitzner et al., 2006). We next investigated if miR-223 is involved in this process. Epidermal single cell suspensions were pulsed with OVA overnight, and then sorted LCs were co-cultured with antigen-specific $CD8^+$ T cells from OT-I mice or $CD4^+$ T cells from OT-II mice for 3 days. LCs from miR-223KO mice showed significantly increased capacity to stimulated antigen-specific $CD8^+$ T cells compared to LCs from WT mice, while LCs from KO and WT mice showed comparable stimulation for antigen-specific $CD4^+$ T cells (Fig. 2A). Furthermore, we performed LC antigen presentation to CD8 Tcells *in vivo*. miR-223KO and WT mice were reconstituted with CFSE-labeled OT-I CD8 T cells, and then challenged with OVA or PBS (as control) that were placed on the shaved and tape-striped back skin of recipient mice. The skin LNs were collected 48h later and analyzed for CFSE staining by flow cytometry to assess the specific proliferation of the adoptively transferred OT-I T cells. As showed in Fig. 2B, increased OT-1 T cell proliferation was also observed in miR-223KO mice compared to WT mice with OVA challenge. As expected, no significant difference was observed in OT-I T cells between miR-223 knockout and WT mice with PBS challenge. Thus, lack of miR-223 increases LC cross-presentation to $CD8^+$ T cells. A recent study indicated that miR-223 is one of the most upregulated miRNAs in human and mouse skin of allergy contact dermatitis (ACD) (Vennegaard et al., 2012), which raises the hypothesis that miR223 might be involved in ACD development. We further investigated the effects of miR-223 on 2,4-dinitrofluorobenzene (DNFB)-induced ACD. Interestingly, lack of miR-233 did not significantly affect ACD development (Fig. 2C).

In conclusion, our results indicated that miR-223 deletion did not interrupt LC development and maturation nor ACD development, but significantly increased LC capacity to induce antigen-specific $CD8^+$ T cell proliferation. Interestingly, LCs from $Dicer^{-/-}$ mice, in which most miRNAs including miR-223 were significantly down-regulated, had comparable capacity to stimulate $CD8^+$ T cells, while $CD4^+$ T cell proliferation was reduced (Kuipers et

al., 2010). The normal $CD8^+$ T cell responses observed in Dicer-deficient LCs could be due to the deletion of both miRNAs that positively and negatively regulate LC cross-presentation. Actually, our recent studies indicated that loss of miR-150 significantly decreased LC-mediated $CD8^+$ T cell proliferation (Mi et al., 2012). miR-223 most likely down-regulates the genes that are important for LC cross-presentation function. The exact mechanisms that lead to LC cross-presentation are not completely understood so far. Recent studies indicated the possible involvement of several pathways in this process, including TLR3/7/9, TAP and Type I IFN (Oh et al., 2011, Stoitzner et al., 2006). However, these genes are not potentially targeted by miR-223. The uncovery of more protein coding genes involved in the cross-presentation process should help us to better understand the mechanisms involved in miR-223-mediated LC cross-presentation. To the best of our knowledge, miR-223 is the first miRNA identified to negatively regulate LC cross-presentation. Although miR-223 is upregulated in human and mouse ACD (Vennegaard et al., 2012), deletion of miR-223 did not block ACD development, suggesting that miR-223 may not be a key regulator for ACD or miR-223 may regulate both ACD enhancer and suppressor. In addition, upregulated miR-223 expression in ACD could be due to a feedback regulation to surprise LC-mediated CD8 T cell proliferation and to control a severe skin inflammation. Further identification of miR-223 downstream target genes involved in LC cross-presentation may illuminate the new molecular mechanisms underlying LC cross-presentation and shed light on new intervention strategies for LCs-related epicutaneous vaccination and allergy.

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References

Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–297. [PubMed: 14744438]

Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med. 1996; 184:2417–2422. [PubMed: 8976197]

Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004; 303:83–86. [PubMed: 14657504]

Cobb BS, Hertweck A, Smith J, O'Connor E, Graf D, Cook T, Smale ST, Sakaguchi S, Livesey FJ, Fisher AG, Merkenschlager M. A role for Dicer in immune regulation. J Exp Med. 2006; 203:2519–2527. [PubMed: 17060477]

Fukao T, Fukuda Y, Kiga K, Sharif J, Hino K, Enomoto Y, Kawamura A, Nakamura K, Takeuchi T, Tanabe M. An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. Cell. 2007; 129:617–631. [PubMed: 17482553]

Heinz LX, Platzer B, Reisner PM, Jorgl A, Taschner S, Gobel F, Strobl H. Differential involvement of PU. 1 and Id2 downstream of TGF-beta1 during Langerhans-cell commitment. Blood. 2006; 107:1445–1453. [PubMed: 16223775]

Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature. 2008; 451:1125–1129. [PubMed: 18278031]

Kaplan DH. In vivo function of Langerhans cells and dermal dendritic cells. Trends Immunol. 2010; 31:446–451. [PubMed: 21035396]

Kissenpfennig A, it-Yahia S, Clair-Moninot V, Stossel H, Badell E, Bordat Y, Pooley JL, Lang T, Prina E, Coste I, Gresser O, Renno T, Winter N, Milon G, Shortman K, Romani N, Lebecque S,

Malissen B, Saeland S, Douillard P. Disruption of the langerin/CD207 gene abolishes Birbeck granules without a marked loss of Langerhans cell function. Mol Cell Biol. 2005; 25:88–99. [PubMed: 15601833]

Kuipers H, Schnorfeil FM, Fehling HJ, Bartels H, Brocker T. Dicer-dependent microRNAs control maturation, function, and maintenance of Langerhans cells in vivo. J Immunol. 2010; 185:400–409. [PubMed: 20530258]

Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003; 115:787–798. [PubMed: 14697198]

Liston A, Lu LF, O'Carroll D, Tarakhovsky A, Rudensky AY. Dicer-dependent microRNA pathway safeguards regulatory T cell function. J Exp Med. 2008; 205:1993–2004. [PubMed: 18725526]

Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol. 2008; 8:935–947. [PubMed: 19029989]

Mi QS, Xu YP, Qi R, Shi Y, Zhou L. Lack of microRNA miR-150 reduces the capacity of epidermal Langerhans cell cross-presentation. Exp Dermatol. 2012; 21:876–878. [PubMed: 23163655]

Nagao K, Ginhoux F, Leitner WW, Motegi S, Bennett CL, Clausen BE, Merad M, Udey MC. Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. Proc Natl Acad Sci U S A. 2009; 106:3312–3317. [PubMed: 19218433]

Oh JZ, Kurche JS, Burchill MA, Kedl RM. TLR7 enables cross-presentation by multiple dendritic cell subsets through a type I IFN-dependent pathway. Blood. 2011; 118:3028–3038. [PubMed: 21813451]

Romani N, Clausen BE, Stoitzner P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol Rev. 2010; 234:120–141. [PubMed: 20193016]

Seo KH, Zhou L, Meng D, Xu J, Dong Z, Mi QS. Loss of microRNAs in thymus perturbs invariant NKT cell development and function. Cell Mol Immunol. 2010

Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, Xu N, Meisgen F, Wei T, Bradley M, Stenvang J, Kauppinen S, Alenius H, Lauerma A, Homey B, Winqvist O, Stahle M, Pivarcsi A. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. J Allergy Clin Immunol. 2010; 126:581–589. e581-520. [PubMed: 20673989]

Stoitzner P, Tripp CH, Eberhart A, Price KM, Jung JY, Bursch L, Ronchese F, Romani N. Langerhans cells cross-present antigen derived from skin. Proc Natl Acad Sci U S A. 2006; 103:7783–7788. [PubMed: 16672373]

Vennegaard MT, Bonefeld CM, Hagedorn PH, Bangsgaard N, Lovendorf MB, Odum N, Woetmann A, Geisler C, Skov L. Allergic contact dermatitis induces upregulation of identical microRNAs in humans and mice. Contact Dermatitis. 2012

Zheng Q, Zhou L, Mi QS. MicroRNA miR-150 is involved in Valpha14 invariant NKT cell development and function. J Immunol. 2012; 188:2118–2126. [PubMed: 22287707]

Zhou L, Park JJ, Zheng Q, Dong Z, Mi Q. MicroRNAs are key regulators controlling iNKT and regulatory T-cell development and function. Cell Mol Immunol. 2011; 8:380–387. [PubMed: 21822298]

Zhou L, Seo KH, He HZ, Pacholczyk R, Meng DM, Li CG, Xu J, She JX, Dong Z, Mi QS. Tie2cre-induced inactivation of the miRNA-processing enzyme Dicer disrupts invariant NKT cell development. Proc Natl Acad Sci USA. 2009; 106:10266–10271. [PubMed: 19509335]

Highlights

- MicroRNA miR-223 is highly expressed in the epidermal LCs;
- miR-223 regulates LCs-mediated $CD8^+$ T cell proliferation.
- miR-223 is not required for the development, maturation, migration and phagocytic capacity of LCs.

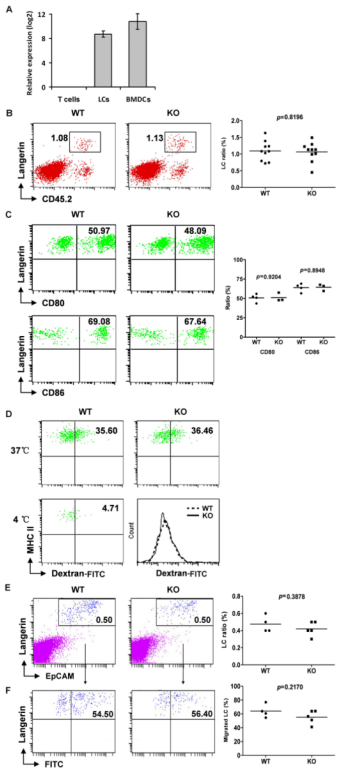

Fig 1. MicroRNA miR-223 is not required for LC development, maturation, phagocytosis, and migration

(A) miR-223 gene expression. Splenic T cells, BMDCs, and enriched epidermal LCs using AutoMACS, were sorted by FACSAria™II Cell Sorter. Total RNA were extracted for miR-223 expression using TaqMan® MicroRNA Assays. snoRNA135 was used as an endogenous control. (B) miR-223 is not required for ontogeny of epidermal LCs. Epidermal suspensions prepared from skin were stained with anti-langerin and anti-CD45.2 antibodies and analyzed by flow cytometry. (C) miR-223 is not an essential factor to control LC maturation. Epidermal cells were cultured in RPMI for 48h, then stained with anti-langerin, CD80, CD86 antibodies and analyzed by flow cytometry. (D) LCs from miR-223KO mice were able to phagocyte FITC-dextran efficiently. Epidermal cells were incubated with 0.25 mg/ml FITC-Dextran for 45min at 4°C (control) or 37°C, and then stained with anti-MHCII and anti-CD45.2 antibodies. The percentage of $FITC^+$ cells in LCs ($CD45.2^+MHCII^+$ cells) was determined. (E) and (F), *In vivo* migration of LCs is unaffected in miR-223KO mice. The mice were painted with 200ul 5mg/ml FITC in acetone/dibutylphthalate. LN cells collected 24hrs later were stained with anti-CD8, anti-Langerin and anti-EpCAM antibodies. The percentages of $Langerin^+EpCAM^+$ LCs were gated on $CD8^-$ cells (E), and the percentages of migrated $FITC^+$ LCs were further analyzed on gated $langerin^+EpCAM^+$ LCs (F). Data are representative of 2–3 independent experiments.

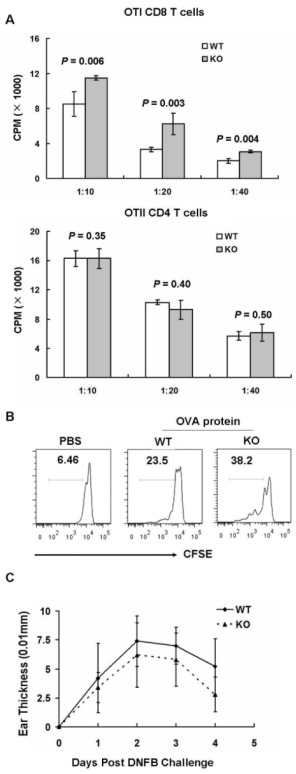

Fig 2. Lack of miR-223 enhances epidermal Langerhans cell cross-presentation, but does not interrupt ACD development

(A) Epidermal cell suspensions prepared from ears and trunk skin of miR-223KO and WT mice were pulsed with 0.5 mg/ml ovalbumin protein overnight. After extensive washing cells were further cultured until day 3, and then enriched LCs by AutoMASC were further sorted by a FACSAria™II sorter. The different ratios of sorted LCs were used to co-culture with 2.5×10^4 antigen-specific OT-I $CD8^+$ T cells and OT-II $CD4^+$ T cells for 56 hours. Incorporation of radioactivity during the last 16 h of culture was measured. Data were representative from 2–3 independent experiments (3–4 mice in each experiment). (B) In vivo OVA presentation. miR-223KO and WT mice were reconstituted with CFSE-labeled OT-I CD8 T cells. After 48h, 50 μl of PBS (as a vehicle control) only or with 25 μg of OVA were painted on the shaved and tape-striped back skin of recipient mice. An additional 48h later, skin LNs were collected and analyzed for CFSE staining by flow cytometry to assess the specific proliferation of the adoptively transferred OT-I T cells. (C) Induction of ACD in mice. Specific ear swelling is shown in different time points during DNFB-induced ACD. They are representative of three experiments with 5–7 mice per group. Mean ± SD values are shown, $p>0.05$.