Endogenous and tumour-derived microRNAs regulate cross-presentation in dendritic cells and consequently cytotoxic T cell function

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Abstract Dendritic cells (DCs) are potent antigen presenting cells (APCs). They are also specialized in the induction of cytotoxic T lymphocyte mediated responses against extracellular antigens, including tumour-specific antigens, by presenting peptide-Major Histocompatibility Complex (MHC) I complexes to naïve CD8⁺ T cells in lymphoid tissues, a process called cross-presentation. Emerging evidence suggests that the efficiency of cross-presentation can be influenced by a unique set of microRNAs (miRNAs). Some are differentially expressed in the course of morphological and functional development of DCs while tumorigenic miRNAs (onco-miRs) can be delivered to and inserted into DCs via exosomes. The latter reprogram the miRNA repertoire of DCs, transforming them from effective APCs to negative modulators of immunity, ultimately aiding cancers to evade host immunity. On the other hand, endogenous microRNAs can influence cross-presentation either positively or negatively. In this review, we discuss the possible mechanisms by which specific miRNAs

influence cross-presentation as well as the viability of manipulating the expression of miRNAs that regulate DC cross-presentation as a potential cancer immunotherapy intervention.

Keywords Cross-presentation · Dendritic cell · Major histocompatibility complex · microRNA

Introduction

Dendritic cells (DCs) are a diverse population of motile leukocytes of stellate morphology found in virtually all body tissues (Steinman 2007). They are the main ‘professional APCs’ in vivo (Merad et al. 2013), and are functionally and anatomically specialized for the uptake, processing, and presentation of antigens to naïve CD4⁺ and CD8⁺ T cells subsequently inducing antigen specific T cell immunity (Banchereau and Steinman 1998). Conversely, non-activated (immature) DCs can present self-antigens to T cells (Heath and Carbone 2001) leading to immune tolerance.

A unique set of highly expressed cell surface molecules called Major Histocompatibility Complex (MHC) intra-cellularly bind peptide fragments derived from antigens, process and display them on the cell surface of DCs for recognition by the appropriate T-cells (Janeway et al. 2001). Initially, MHC II molecules were understood to present peptides derived

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from extracellular antigens to CD4⁺ T cells while MHC I molecules were thought to present peptides of intracellular origin to CD8⁺ T cells (Rock et al. 2002). However, Bevan first recognized that DCs can present extracellular antigens to CD8⁺ T cells via an MHC I restricted pathway (Bevan 1976), a phenomenon called cross-presentation (Yewdell et al. 1999) which has the advantage of channelling extracellular antigens of pathogenic or tumour origin to effector CD8⁺ T cells without them directly infecting APCs (Nair-Gupta and Blander 2013). Subsequently, there is T cell activation, proliferation and acquisition of cytotoxic functions against infected or malignant target cells (Villadangos et al. 2007; Platzer et al. 2014) a process called cross-priming. Several miRNAs are implicated as regulating specific steps in this cascade of events.

The ability of DCs to cross-present is directly related to what maturation state they are in, and by what phenotype they characterize (e Sousa 2006). Specialized subsets of immature DCs exhibit excellent antigen uptake capabilities but poor CD8⁺ T cell stimulatory qualities. The converse is also true for mature DCs (Mellman and Steinman 2001). Therefore, stimuli that induce maturation of antigen pulsed immature DCs ultimately promote cross-presentation. It is also worth noting that both cross-presentation and cross-priming are complex pathways that depend on a number of factors for optimum induction of CD8⁺ T cell mediated immunity.

Dendritic cell subtypes

In both humans and mice DCs develop from hematopoietic precursors in bone marrow called macrophage and DC precursors (MDP) (Fogg et al. 2006) that eventually differentiate into two major subsets. Plasmacytoid DCs (pDC) terminally differentiate in the bone marrow (Reizis 2010) while the second subset exits as pre-DCs which migrate through blood to lymphoid and non-lymphoid tissue where they differentiate into myeloid or conventional DCs (cDC) (Naik et al. 2007; Onai et al. 2007). In human blood, DC sub-types can be further distinguished by the differential expression of three cell-surface molecules: CD303, CD1_C and CD141 (Dzionek et al. 2000). CD303⁺ pDCs perform a key role in anti-viral immunity due to their outstanding capability to secrete IFN α (Siegal et al. 1999) as well as their

ability to rapidly activate initial CD8⁺ T cell response to virus encounters.

Human cDCs can be further discriminated by reciprocal expression of CD1_C and CD141. Murine cDCs too can be classified into CD8 α ⁺ in lymphoid organs and their CD103⁺ counterparts in non-lymphoid tissues (Hashimoto et al. 2011). CD141⁺ DCs and CD8⁺ DCs are superb cross-presenters of both soluble and cell associated antigens (Bachem et al. 2010). In addition, they exhibit similar protein expression profiles, for example XCR1 (Bachem et al. 2010; Crozat et al. 2010), NECL2 (cellular adhesion molecule-1; CADM1) (Galibert et al. 2005) and CLEC9A (Caminschi et al. 2008; Huysamen et al. 2008). They also respond to TLR stimulation in similar fashion (Jelinek et al. 2011). Gene ablation studies in mice show that the CD8⁺ DCs have an important role in cross-presentation (Shortman and Heath 2010).

The human skin hosts two main mDC subtypes: epidermal Langerhans cells and dermal interstitial DCs (dermal DCs) (Valladeau and Saeland 2005). The dermal DCs can be further divided into CD1a⁺ DCs and CD14⁺ DCs (Nestle et al. 1993), the latter being specialized for the development of humoral responses (Ueno et al. 2007; Klechevsky et al. 2008). On the other hand Langerhans cells are highly efficient cross-presenters (Klechevsky et al. 2008) and can prime the differentiation of CD8⁺ T cells into effector CTLs. Other DC subtypes that can also cross-present effectively include lung and dermal CD103⁺ DCs as well as monocyte-derived inflammatory DCs (Segura and Villadangos 2009; Shortman and Heath 2010). Spectacular cross-presentation capability can be attributed to, at least in part to the low levels of lysosomal proteases in intracellular endocytic pathways (Delamarre et al. 2005) as well as the maintenance of high pH levels (7.5–8), that inhibit proteolytic activity (Claus et al. 1998) during migration from tissues to lymph nodes (LNs).

Mechanism of cross-presentation

The exact mechanism of cross-presentation is not yet well understood. However Cytotoxic T Cells (CTLs) require antigen specific activation by mature DCs through cell to cell contact mediated by MHC I-antigen complexes, co-stimulatory molecules (e.g.

Fig. 1 Dendritic cells present extracellular antigen derived peptides to naïve $CD8^+$ T cells. Quality activation of T cell responses requires interaction between MHC I-peptide complexes as well as co-stimulatory molecules and IL-12 secretion by DCs

CD80 and CD86) and cytokine production for instance interleukin-12p70 (IL-12p70) for full expression cytotoxic functions (Keller et al. 2009; Robson et al. 2010). Figure 1 shows the interaction between mature DCs and effector T cells during cross-presentation.

Nevertheless, the intracellular mechanisms of cross-presentation are better understood. Antigens are captured by immature DCs and internalized via clathrin-mediated endocytosis, phagocytosis, or macropinocytosis depending on the nature of antigen (Blum et al. 2013). Endocytosis cell surface receptors exclusively expressed by cross-presenting cells, such as Mannose Receptor (MR) (Burgdorf et al. 2007) and DEC 205 can either capture or promote the internalization of antigens into DCs and subsequently aid in directing these antigens towards MHC I as opposed to MHC II loading (Burgdorf and Kurts 2008). Noting that some $CD8^-$ DCs can also cross-present, endocytosis cell surface receptor expression may be the more useful identifier of cross-presenting APCs as compared to CD8 expression (Burgdorf and Kurts 2008).

Two MHC-I restricted pathways are confirmed. In the vacuolar pathway, also called TAP independent cross-presentation, internalized proteins are degraded by endosomal proteases, mainly cathepsin S, and the resultant peptides loaded onto MHC I within endosomes (Nair et al. 2011; Nair-Gupta and Blander 2013). In TAP dependent cross-presentation or the cytosolic pathway, antigens are initially internalized

into phagosomes. The NADPH oxidase NOX2 actively alkalizes the phagosome thus restricting the activation of proteases (Savina et al. 2006) that could potentially cause their rapid degradation (Jancic et al. 2007). The antigens are then translocated to the cytosol prior to degradation by proteasomes. Resulting peptides are then transported back into phagosomes via TAP for MHC-I loading (Cebrian et al. 2011; Joffre et al. 2012).

Upon uptake of cargo, the phagosomal or endosomal compartments can be substantially modified by the acquisition of pattern recognition receptors such as TLRs (Schaible et al. 2003). TLRs recognize and respond to non-self molecules called pathogen-associated molecular patterns (PAMPs) (Blander and Sander 2012) and subsequently initiate signalling to regulate adaptive immune responses such as cross-presentation (Nair et al. 2011; Nair-Gupta and Blander 2013). Downstream signalling may be mediated by proteins such as nuclear factor- κ B (NF- κ B), mitogen-activated protein kinases (MAPKs), and IFN-regulatory factors (IRFs) (Takeuchi and Akira 2010).

Several studies reported that TLR ligands could induce cross-presentation of OVA by murine DCs as measured by T cell activation (Maurer et al. 2002; Datta et al. 2003). Considering that TLR signalling controls both DC maturation and expression of co-stimulatory molecules that are key to $CD8^+$ T cell activation, it is difficult to determine whether TLR signalling influences actual cross-presentation, co-stimulatory molecule expression or both the regulation of downstream TLR4 signalling by miRNAs is demonstrated in Fig. 2.

microRNAs

miRNAs form a class of small endogenous non-coding single strand regulatory RNAs around 19–24 nucleotides long (Bartel 2009; Krol et al. 2010).

They are important regulators immune cell differentiation, function and fate (Zhou et al. 2011; Zheng et al. 2012). In humans and mice the differential expression of over 300 miRNAs in the course of differentiation of DCs is well documented (Turner et al. 2011; Su et al. 2013) and their influence on DC function is demonstrated by in vivo gain or loss-of-function studies (Xiao and Rajewsky 2009). Nevertheless, the causality between findings from gain or

Fig. 2 Regulation of TLR-signalling pathways by miRNAs. miRNAs are known or presumed to target several molecules along these pathways, including TLRs themselves, TLRs-associated signalling proteins, TLR-associated regulatory molecules, TLR-induced transcription factors, and TLR-induced functional cytokines, ultimately influencing cross-presentation by DCs

loss-of-function studies involving miRNAs should be interpreted with caution since complex variables may be at play (Reichel et al. 2011).

miRNAs alter gene expression through translational inhibition or accelerating deadenylation and degradation of target mRNAs (Huntzinger and Izaurralde 2011; Stroynowska-Czerwinska et al. 2014) and can control cross-presentation, in addition to regulating cytokine production and co-receptor levels in DCs (Smyth et al. 2015) the latter two having a bearing on cross-presentation efficiency. As a result, manipulating miRNA expression, can alter DC function in both humans and mice (Hashimi et al. 2009; Kuipers et al. 2010). miRNAs may be intrinsic to DCs or may originate from tumours (Mittelbrunn et al. 2011; Montecalvo et al. 2012).

miRNA precursors are sequentially processed by two enzymes, Drosha (Kim and Kim 2007; Xiao and

Rajewsky 2009) and Dicer (Kok et al. 2007; Koscińska et al. 2011). Mature miRNAs are then integrated into the RNA-induced silencing complex (RISC). When post-transcriptionally paired with mRNAs containing motifs that are complementary to the miRNA sequence the target genes are silenced.

Deletion of Dicer is used to test the relevance of miRNAs in DC function, and numerous miRNA were declared functionally redundant on this basis (Mi et al. 2013). However, other biosynthesis pathways may exist following description of Dicer independent (Cifuentes et al. 2010; Yang et al. 2010) and Drosha independent (Ruby et al. 2007) pathways.

Tumour derived miRNAs are transferred to DCs via exosomes which are actively secreted through an exocytosis pathway (Clayton et al. 2007). Exosomes can deliver their cargo into DCs during cross-priming

of T cells, or during the differentiation of DCs from monocytes (Iero et al. 2008).

Tumour derived miRNAs regulate cross-presentation in DCs

miR-203

In humans, miR-203 is over expressed in pancreatic adenocarcinomas in comparison with normal pancreatic cells and is implicated in the progression of this cancer (Bloomston et al. 2007). This miRNA was confirmed as constituting the cargo in exosomes secreted by panc-1 cells in vitro (Zhou et al. 2014).

Previous reports suggested that TLR4 was the target of miR-203 mediated regulation of DC function (Szafranska et al. 2007; Greither et al. 2010). Zhou and colleagues reported a reciprocal relationship between miR-203 and TLR4 expression in human peripheral blood mononuclear cells (PBMCs). However, mRNA levels of TLR 4 were not significantly altered despite the existence of partial sequences complementary between miR-203 and its binding site suggesting that inhibition likely took place at the translational level. Christian Kurts and colleagues had earlier established that cross-presentation was enhanced with TLR4, MyD88 signalling following the uptake of soluble ovalbumin (OVA) in presence of the TLR4 ligand lipopolysaccharide (LPS) (Burgdorf et al. 2008). Nevertheless, whether TLR signalling enhances cross-presentation of phagocytized particulate antigen is yet to be confirmed.

In the Zhou et al. report, cross-presentation of pancreatic cancer antigens by DCs was assayed indirectly where they noted a significant depression in production of tumour necrosis factor- α (TNF- α) and IL-12 by DCs transfected with miR-203 mimics which was reversed by inhibitors. Notably, activation of NF- κ B initiated by TLR4 is involved in producing cytokines such as TNF- α and IL-12 (Akira and Takeda 2004; Huang et al. 2008). At low concentrations, TNF- α is a potent DC maturation promoter that enforces cross-presentation ability of DCs while IL-12 production is usually upregulated in mature DCs and plays a key role in CD8⁺ T cell priming. Therefore, ectopic miR-203 inhibition may find application in management of pancreatic cancer.

Tumours influence expression of endogenous miRNAs that regulate cross-presentation in DCs

miR-301a

In humans a specific subset of migratory lymph node DCs (migr-LNDCs) exclusively cross-presents tumour-antigens derived from intrapulmonary growing tumours to effector T cells in the mediastinal LNs (Desch et al. 2011).

Pyfferoen and colleagues reported that in vivo murine miR-301a expression was modulated in the presence of lung tumours. Transient transfection of LNDCs with miR-301a-specific mimics resulted in a striking repression of IFN- γ release from antigen-specific CD8⁺ responders. TNF- α production by migr-LNDCs was also repressed, but was reversed by miR-301a inhibitors. They further reported that Dicer-insufficient mice showed a significant survival advantage and lower pulmonary metastatic tumour load over the control group. However, DC-driven CD4⁺ and CD8⁺ T cell proliferation was not altered by miR-301a overexpression in DCs (Pyfferoen et al. 2014).

Taken together, the findings of Pyfferoen and colleagues suggest that tumour-exposed migr-LNDCs overexpress miR-301a. The resulting depression of TNF- α production suggests an impaired ability of migr-LNDCs to mature and therefore effectively cross-present lung tumour antigens and subsequently induce quality effector T cell responses. IFN- γ —an indicator of acquisition cytotoxic activity produced by T cells too was diminished. This paradigm was corroborated by the improved therapeutic outcomes in mice when miR-301a biosynthesis was blocked. However, they also reported that T cell proliferation was not significantly changed by forced expression of miR-301a. This could be because predominantly for CD8⁺ T cells, production of cytokines like IL-12 and type 1 IFNs by DCs directs the acquisition of effective cytotoxic functions versus tolerance, irrespective of DC capacity to induce proliferation (Curtsinger et al. 2003). Therefore, the inhibition of miR-301a expression in DCs may be beneficial in lung cancer patients.

miR-301a is known to amplify STAT3 activity by targeting its negative regulator, PIAS3 (Mycko et al. 2012). STAT3 signalling directs an immunosuppressive cascade that emanates from cancerous cells to dendritic cells, inhibiting of DC maturation and

profoundly depressing IL-12 production (Wang et al. 2004).

miR-146a

miRNA-146a is thought to be associated with the regulation of DC differentiation, maturation and function (Chen et al. 2011). The up-regulation of miR-146a causes a direct Notch1 repression (a known positive regulator of IL-12p70 production in DCs) and subsequent reduction of IL-12p70 production in DCs (Bai et al. 2012).

Pancreatic cancer cells are known to induce tolerogenic DC phenotypes (Bellone et al. 2006). Therefore, in culture media conditioned with an extremely metastatic human pancreatic cancer cell line BxPC-3(BxCM) Du and colleagues found that BxCM-treated human iDCs and matured with TNF- α showed a dramatic dose-dependent repression of DC maturation markers CD80, CD86 and CD1a in mDCs compared with normal mDCs. Strikingly, in BxCM-treated DCs the expression levels of miR-146a were notably up-regulated with a corresponding depression of the protein Smad 4 in both iDCs and mDCs, compared with untreated DCs. They further used human pancreatic cancer antigen-loaded BxCM-treated mDCs versus normal mDCs to compare their effect on activation of autologous T cells, T cell proliferation and cytotoxic T cell response against BxPC-3 cells. As expected they reported a significant suppression of IFN- γ production by T cells, T cell proliferation and CTL responses in the in BxCM group. They also noted a partial reversal of these effects when the mDCs were transfected with miR-146a inhibitors (Du et al. 2012).

From the above study we can conclude that pancreatic cancers can suppress monocyte-derived DC maturation and functions, including cross-presentation, findings that are consistent with earlier works by Chen et al. (2011), partly by up-regulating miR-146a expression level in DCs. miR-146a's negative effects appear to be mediated via Smad4 dependent signalling pathway and is achieved by impairing DC maturation, and induction of T cell responses against pancreatic cancers. Considering that these effects are partly reversible by miR-146a inhibitors, miR-146a might be a feasible molecular target for pancreatic cancer immune-therapy.

miRNAs regulate cross-presentation in Langerhans cells

Epidermal Langerhans cells (LCs) are Langerin⁺ EpCAM⁺ DCs (Nagao et al. 2009). They are excellent cross-presenters, compared to interstitial DCs also resident in skin, (Palucka and Banchereau 2012) of soluble exogenous ovalbumin (OVA) as well as cellular antigens from the epidermis (Kissenpfennig et al. 2005; Stoitzner et al. 2006).

LCs are of special interest to us because the deletion of Dicer in DCs only affects the homeostasis and function of LCs, but not other types of DCs (Kuipers et al. 2010; Xu et al. 2012) giving a strong indication that miRNAs regulate LC development and function.

miR-223

Mi and colleagues first reported that miR-223 is highly expressed in freshly isolated epidermal LCs. They further studied the effect of miR-223 on DC maturation and function using Langerin⁺ CD45.2⁺ cells obtained from either miR-223 KO or WT mice. Their findings showed that miR-223 is not critically required for LC development (Kuipers et al. 2010) and maturation as well as antigen capture and migration to draining LNs.

However, when Langerin⁺ CD45.2⁺ cells pulsed with soluble OVA and co-cultured with antigen-specific CD8⁺ T cells from OT-I mice LCs from miR-223 knockout (KO) mice showed significantly increased capacity to stimulate antigen-specific CD8⁺ T cells compared to LCs from wild type (WT) mice. Similar results were observed with in vivo experiments (Mi et al. 2013). It therefore appears that miR-223 most likely down-regulates the genes that are important for LC cross-presentation. Interestingly, LCs from Dicer^{-/-} mice, in which most miRNAs including miR-223 were significantly down-regulated, had comparable capacity to stimulate CD8⁺ T cells compared with WT mice. This is probably because deletion of Dicer down regulates both miRNAs that positively and negatively regulate LC cross-presentation.

The genes targeted by miR-223 are still unknown. So are the exact mechanisms that lead to LC cross-presentation. However, studies point towards the possible involvement of several pathways, including TLR 3/7/9, TAP and Type I IFN (Stoitzner et al. 2006; Oh et al. 2011).

However, the miR-223 gene is likely driven by PU.1, and PU.1 depression down-regulates miR-223 expression (Fukao et al. 2007). PU.1 is in turn regulated by transforming growth factor- β 1 (TGF- β 1), TGF- β 1 induces PU.1 expression in CD34⁺ hematopoietic progenitor cells undergoing LC commitment, and ectopic expression of PU.1 strongly enhanced TGF- β 1-dependent LC development (Heinz et al. 2006). TGF- β 1 is critical in the differentiation of LCs from their precursors both in vitro and in vivo (Merad et al. 2008; Romani et al. 2010). It therefore follows, at least in part, that the TGF- β 1- PU.1- miR-223 cascade may play a role in regulating the functional capability of epidermal LCs to cross-present antigens.

miR-150

Qing-Sheng Mi and colleagues found that miR-150 is expressed in freshly-isolated epidermal LCs, even though the expression level is lower than that of bone marrow-derived DCs. Most interestingly, expression level of miR-150 was significantly down-regulated (threefold) during in vitro LC maturation.

When matured, LCs highly express co-stimulatory molecules including CD80, and CD86, which are required for the stimulation of CD8⁺ T cells via cross-presentation (Kaplan 2010; Romani et al. 2010). The frequencies of CD86⁺ and CD80⁺ expression LCs in freshly-prepared LCs are comparable between miR150 KO and WT mice, suggesting that lack of miR-150 does not promote the spontaneous maturation of LCs in vitro. Similar results were noted with survival and homeostasis, antigen capture and migratory capacity to LNs (Busch and Zernecke 2012).

In contrast, when epidermal LCs pulsed with soluble ovalbumin were co-cultured with antigen-specific CD8⁺ from OT-I mice Mi et al. noted a significantly decreased capacity of stimulating antigen-specific CD8⁺ T cell proliferation and IFN- γ by miR-150 KO mice compared to LCs from WT mice. Interestingly, their capacity of stimulating antigen specific CD4⁺ T cells was not significantly changed. These findings suggest that miR-150 disrupts cross-presentation of soluble antigen to antigen-specific CD8⁺ T cells (Mi et al. 2012). However, miR-150 target genes are yet to be identified and neither are the exact cross-presentation mechanisms in LCs. For similar reasons to miR-223 above, LCs from Dicer^{-/-} mice, in which most miRNAs including

miR-150 were significantly down-regulated, had comparable capacity to stimulate CD8⁺ T cells compared with WT mice.

Differential expression of miRNAs in DCs in general and possible role in cross-presentation

miR-155

miR-155 is quite versatile and has been implicated in regulation of maturation of both mouse and human moDCs, cytokine production and expression of co-stimulatory molecules. miR-155 also plays an important role in oncogenesis and is highly expressed in many solid tumours (Xiao and Rajewsky 2009).

miR-155 expression is highly upregulated upon maturation of multiple subsets of mouse and human cDCs (Zhou et al. 2010; Dunand-Sauthier et al. 2011). Despite this, several authors suggested that it has no role in DC antigen presentation (Zhou et al. 2010; Turner et al. 2011). Bone marrow-derived DCs (BM-DCs) from miR155^{-/-} mice are impaired in their ability to activate T cells but the authors reported a possible role of miR-155 in MHC II restricted antigen presentation (Rodriguez et al. 2007) but not cross-presentation.

Nevertheless, a recent study reported that ectopic supplementation of miR-155 robustly enhanced tumour associated DC mediated T cell responses. In vitro, ovarian cancer associated mice DCs and transfected with miR-155 mimetics were pulsed with soluble OVA and co-cultured with OT-1 cells. In vitro, they reported enhanced proliferation of CD8⁺ T cells, amplified number of CD8⁺ T cells exhibiting central memory attributes and increased number of antigen experienced CD8⁺ T cells, suggesting that cross-presentation and cross-priming actually took place. In vivo experiments showed an increase in production of cross-presentation associated cytokines, TNF- α , IL-12 and IFN- γ . miR-155 seemed to depress expression of genes in tumour associated DCs such as c/ebp β (Cubillos-Ruiz et al. 2012). Therefore miR-155 may play a role in cross-presentation. The investigators noted that endogenous processing of synthetic miR-155 mimetics may result in genome-wide transcriptional changes that include silencing of multiple immunosuppressive mediators. In addition, a miRNA hierarchy directed by miR-155 either stimulates or

represses the expression of over fifteen other miRNAs (Dueck et al. 2014) some of which possibly play a role in cross-presentation.

Overall transfection of tumour bearing mice with miR-155 mimics resulted in arrested disease progression and significantly increased survival (Cubillos-Ruiz et al. 2012). Therefore, forced expression of miR-155 may find application in DC based cancer immunotherapy.

miR-24, miR-30b and miR-142-3p

While investigating the influence of miR-24, miR-30b and miR-142-3p on phagocytosis of antigens Naqvi and colleagues reported that human DCs transfected with mimics of these miRNAs showed a 4–6 folds decrease in phagocytic uptake of *E. coli*. Transfection inhibitors showed no significant effect compared to controls (Naqvi et al. 2015).

In another study, the investigators reported that overexpression of miR-30b or miR-142-3p resulted in a significant decrease in TLR4 expression in mD-DC. The DCs were further challenged with LPS, a known depressor of TLR 4 expression and TLR4 levels assayed a day later. In mD-DC, transfection with miR-30b and miR-142-3p inhibitors significantly reduced LPS induced depression of TLR4 expression. The

corresponding mimics had the opposite effect but the results were not statistically significant.

PKC α is a component of GM-CSF receptor signalling and subsequently DC differentiation as well as PRR-mediated activation (Guler et al. 2011). While studying miRNA regulation of PKC α the authors found that miR-30b, and miR-142-3p mimics reduced PKC α activation relative to controls. They also noted that overexpression of miR-24, miR-30b, and miR-142-3p mimics decreased NF-kBp65 activation in mD-DC. miR-142-3p had the most profound effects on NF-kBp65 levels. Their corresponding inhibitors had no significant effect. As expected forced expression of these miRNAs in LPS stimulated DCs resulted in suppression of the cytokines TNF- α and IL-12 (p40) (Fordham et al. 2015).

Taken together, these three findings indicate that these miRNAs negatively regulate the differentiation, activation antigen capture and internalization, as well as intra-cellular signalling in DCs (Mycko et al. 2012). All these processes have some influence on the ultimate cross-presentation efficiency of DCs. The investigators also noted that these miRNAs appear to have multiple targets, among them PKC α and NF-kB.

Table 1 summarises the miRNAs that are thought to regulate cross-presentation by DCs.

Table 1 Summary of miRNAs presumed or confirmed to influence cross-presentation in DCs

miRNA	Origin	Presumed/confirmed target	Outcome	References
miR-203	Pancreatic adenocarcinoma	TLR4	↓	Zhou et al. (2014)
miR-301a	DCs in intrapulmonary growing tumours	PIAS3	↓	Mycko et al. (2012)
miR-223	Epidermal LC	TLR4 IKK α	↓	Mi et al. (2013)
miR-150	Epidermal LC	?	↑	Mi et al. (2012)
miR-155	DC/synthetic pre-miR155 mimetic	c-Fos c/epb β	↑	Marigo et al. (2010), Dunand-Sauthier et al. (2011), Cubillos-Ruiz et al. (2012)
miR146a	DC	Smad4	↓	Du et al. (2012)
miR-142-3p	DC	PKC α NF-kB	↓	Fordham et al. (2015)
miR-30b	DC	PKC α NF-kB	↓	Fordham et al. (2015)
miR-24	DC	PKC α NF-kB	↓	Fordham et al. (2015)

Conclusion

Current evidence suggests that miRNAs regulate actual cross-presentation by DCs as well as the related cellular processes that are vital in conferring upon DCs their outstanding cross-presentation capabilities.

Since DCs can ardently take up miRNAs (Cubillos-Ruiz et al. 2009) from their environment, miRNA manipulation can find clinical application whereby inhibitors can be inserted into DCs to disrupt miRNA:mRNA interactions thus achieving a loss of function effect (Reichel et al. 2011) while mimetics can achieve a gain of function effect (Cubillos-Ruiz et al. 2012). In contrast, tumours themselves are not viable targets of miRNA intervention due to poor uptake, low bioavailability and copious phagocytic activity of immune cells in the tumour microenvironment (Garzon et al. 2010).

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