Published in final edited form as:

Nat Immunol. 2014 January ; 15(1): 8–9. doi:10.1038/ni.2786.

A new VEGF connection between two old neighbors

Marina Cella, Giorgio Trinchieri

Marina Cella is with the Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, USA., Giorgio Trinchieri is with the Cancer and Inflammation Program, National Cancer Institute, Frederick, Maryland, USA.

Abstract

Plasmacytoid dendritic cells express the microRNA miR-126, which controls their survival and effector functions via the receptor VEGFR2.

Plasmacytoid dendritic cells (pDCs) are a unique DC subset characterized by robust production of type I interferons in response to viruses and bacteria. That response is triggered by the recognition of microbes by Toll-like receptor 7 (TLR7) or TLR9, which bind single-stranded RNA or double-stranded DNA, respectively¹. In this issue of *Nature Immunology*, Agudo *et al.* identify a microRNA (miRNA), miR-126, that is selectively expressed in pDCs, in which it regulates cell survival and function². miR-126 has been shown to be expressed predominantly by endothelial cells and to regulate angiogenesis³, but the contribution of miR-126 to immune responses was not previously known. Intriguingly, this study shows that lymphoid tissue–resident pDCs and endothelial cells exhibit a common dependence on the signaling pathway of the growth factor VEGF, which is regulated by miR-126.

miRNAs are evolutionary conserved single-stranded, small noncoding RNAs that regulate gene expression at the post-transcriptional level by binding to target sites in the 3' untranslated region (UTR) of target mRNA and either inhibit the translation of mRNA or promote the degradation of mRNA. Through that tight regulation of protein expression, miRNAs control a variety of physiological and pathological processes, including the development and regeneration of organs, cell-lineage specification and the formation and progression of tumors⁴.

Emerging evidence indicates that miRNAs are involved in fine-tuning inflammatory responses to incoming pathogens orchestrated by the innate TLRs and Nod-like receptors (NLRs), as well as in modulating sterile inflammation and chronic inflammatory diseases⁴. Although miRNAs are traditionally considered suppressors of protein expression, they can also promote it, particularly in inflammatory cells. miRNAs may indirectly boost the mRNA expression of off-target genes by repressing the products of regulatory genes. Also, both miRNAs and RNA-binding proteins such as tristetraprolin can regulate AU-rich elements

mcella@pathology.wustl.edu.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

located in the 3' UTR of mRNAs that encode inflammatory mediators. miRNAs can either act together with or antagonize the ability of tristetraprolin to induce the rapid degradation of mRNAs and thus result in either the suppression of or enhancement of protein expression.

A particularly important aspect of miRNAs in the regulation of inflammation and immunity is their role in modulating the development and maturation of DCs. DCs orchestrate the immune response by bridging the innate and adaptive arms of the immune system⁵. Several miRNAs, identified in a published study of DCs generated *in vitro* from bone marrow cells stimulated with the cytokine Flt3L, are selectively expressed by pDCs rather than conventional DCs⁶. However, the biological relevance of that miRNA signature to the development and effector functions of pDCs has not been explored.

The study by Agudo *et al.* shows that miR-126 controls the number of pDCs in secondary lymphoid organs by enhancing the survival of pDCs². pDCs that lack miR-126 also have less production of type I interferons, inflammatory chemokines and cytokines than do wild-type control cells in response to agonists of TLR7 and TLR9 *in vitro* and *in vivo*. Blunted production of type I interferons in the absence of miR-126 is not a mere consequence of a lower abundance of pDCs but also of the decreased intrinsic ability of pDCs to produce type I interferons. miR-126 deficiency in pDCs decreases the expression of many genes along the TLR7- or TLR9-transcription factor NF- κ B pathway, which are involved in cytokine production. Deficiency in miR-126 in pDCs also results in increased expression of TSC1, a negative regulator of the metabolic checkpoint kinase mTOR pathway. The phosphatidylinositol-3-OH kinase (PI(3)K)-kinase Akt-mTOR pathway it is a well-established signaling axis that senses nutrient availability directly and indirectly through growth-factor signaling and thus modulates the proliferation and survival of many cell types. In pDCs, mTOR signaling favors survival and acts together with TLR signaling in the efficient induction of type I interferons, which suggests that the phenotype of miR-126-deficient pDCs is mediated at least in part by the PI(3)K-Akt-mTOR axis. Notably, defective secretion of type I interferons from pDCs that lack miR-126 results in extensive infection of lymph node cells after delivery of vesicular stomatitis virus-pseudotyped HIV *in vivo*², which indicates that loss of miR-126 reprograms the intrinsic pathogen-sensing ability of pDCs (Fig. 1).

The most unexpected finding of the report by Agudo *et al.* is that miR-126 promotes expression in pDCs of the gene that encodes VEGFR2 (*Kdr*)². That tyrosine kinase receptor binds members of the VEGF family of cytokines and promotes angiogenesis in health and disease⁷. The authors show that in both mice and humans, VEGFR2 is expressed by pDCs that have migrated into the tissues but not in pDCs isolated from peripheral blood or bone marrow. The observation that ablation of VEGFR2 in pDCs results in a phenocopy of miR-126 deficiency suggests that VEGF provides prosurvival signals to pDCs. The common expression and function of VEGFR2 in pDCs and endothelial cells it is of particular relevance, because pDCs have been known for long time to accumulate around high endothelial venules (HEVs) in chronically inflamed lymph nodes from patients infected with *Mycobacterium tuberculosis* or affected by Kikuchi's disease. pDCs and HEVs express several common markers, such as CD36 (a pattern-recognition receptor), CD123 (the receptor for interleukin 3) and CD62L (a lymph node-homing receptor)⁸. The revelation that

those two cell types similarly express VEGFR2 and respond to VEGF challenges the view that the connection between pDCs and HEVs is only physical proximity and raises the question of whether a functional relationship also exists between pDCs and HEVs. Of note, the authors report defective inflammation-driven enlargement of the peripheral draining lymph nodes in miR-126-deficient mice infected with vesicular stomatitis virus–pseudotyped HIV. During an immune response, lymph nodes exhibit increases in vascularity due to proliferation and progressive lengthening and arborization of HEVs. That process is necessary for the provision of nutrients for incoming lymphoid and myeloid cells to support an effective immune response. A published study has indicated that remodeling of lymph nodes after immunization involves VEGF and that DCs, broadly defined as CD11c⁺ cells, are needed for an increase in VEGF concentrations⁹. The study by Agudo *et al.*² now raises the intriguing possibility that the CD11c⁺ cells required for the enlargement of lymph nodes and for blood vessel growth are actually pDCs.

pDCs are present in many cancers, including melanoma, breast cancer and the malignant ascites of ovarian carcinomas. Although the precise role of pDCs in cancer is still controversial, in general their presence in the tumors has been interpreted as being immunosuppressive and tumor promoting^{10,11}. In particular, one study has indicated that pDCs selectively recruited to the ascites of ovarian cancer support angiogenesis via the production of tumor-necrosis factor and interleukin 8 (ref. 12). It is conceivable that signaling via VEGF in pDCs might also favor carcinogenesis by endowing pDCs with proangiogenic properties or by inducing the release of other as-yet-unknown factors. If that were indeed the case, depletion of pDCs in tumors could represent an alternative strategy for effectively reducing tumor growth and metastasis, perhaps with fewer side effects than general antagonism of VEGF.

In conclusion, the study by Agudo *et al.* suggests that modulation of miR-126 expression in pDCs may have ‘yin-yang’ effects, depending on the context². Enforcing miR-126 expression during an ongoing immune response to pathogens, such as during acute infection with HIV, may have beneficial effects by boosting pDC-derived type I interferons and the secretion of inflammatory chemokines and, possibly, by promoting pDC-mediated enlargement of lymph nodes to support the efficacy of the immune response. In contrast, sustaining miR-126 expression in pDCs in malignancies might have deleterious effects and promote the growth and dissemination of cancer, perhaps by inducing vessel sprouting and neovascularization. Whereas this elegant study suggests that pDCs and endothelial cells may be more than just ‘friendly neighbors’ and share at least a VEGF-mediated signaling pathway for survival², full understanding of the effect of VEGF signaling on pDC function and conclusive demonstration that pDCs may have angiogenic properties are still missing. Collectively, the study by Agudo *et al.*² and three published studies investigating the effect of selected ablation of miRNAs on pDCs^{13–15} have shown that miRNAs fine tune pDC fate in both the steady state and after pathogen encounter.

References

1. Colonna M, Trinchieri G & Liu YJ Nat. Immunol 5, 1219–1226 (2004). [PubMed: 15549123]
2. Agudo J et al. Nat. Immunol 15, 54–62 (2014). [PubMed: 24270517]

3. Fish JE & Srivastava D *Sci. Signal* 2, pe1 (2009).
4. Lodish HF, Zhou B, Liu G & Chen CZ *Nat. Rev. Immunol* 8, 120–130 (2008). [PubMed: 18204468]
5. Banchereau J & Steinman RM *Nature* 392, 245–252 (1998). [PubMed: 9521319]
6. Kuipers H, Schnorfeil FM & Brocker T *Mol. Immunol* 48, 333–340 (2010). [PubMed: 20822813]
7. Coultas L, Chawengsaksophak K & Rossant J *Nature* 438, 937–945 (2005). [PubMed: 16355211]
8. Facchetti F et al. *Immunol. Lett* 20, 277–281 (1989). [PubMed: 2785501]
9. Webster B et al. *J. Exp. Med* 203, 1903–1913 (2006). [PubMed: 16831898]
10. Labidi-Galy SI et al. *OncoImmunology* 1, 380–382 (2012). [PubMed: 22737622]
11. Vermi W et al. *J. Pathol* 200, 255–268 (2003). [PubMed: 12754747]
12. Curiel TJ et al. *Cancer Res* 64, 5535–5538 (2004). [PubMed: 15313886]
13. Zhou H et al. *Blood* 116, 5885–5894 (2010). [PubMed: 20852130]
14. Karrich JJ et al. *Blood* 122, 3001–3009 (2013). [PubMed: 24014244]
15. Hong Y et al. *PLoS ONE* 8, e69926 (2013). [PubMed: 23894561]

Marina Corral Spence

Figure 1.

A model for miR-126-orchestrated regulation of pDC effector functions in lymph nodes undergoing an immune response. miR-126 expressed in pDCs promotes VEGFR2 expression and VEGF-mediated signaling while blocking TSC1, a negative regulator of the mTOR pathway. Loss of miR-126, by reducing mTOR signaling in pDCs, decreases pDC survival and the expression of genes along the TLR–NF- κ B pathway that drive the production of type I interferon (IFN). Reduced secretion of type I interferon results in viral spreading and dissemination. VEGF also acts on endothelial cells by promoting angiogenesis and vessel sprouting. The identification of cells that produce VEGF in lymph nodes during an immune response and whether pDCs directly contribute to endothelial proliferation or vascular remodeling remain to be delineated. IRF7, transcription factor; MyD88, adaptor; CpG, oligodeoxynucleotide.