Metabolic–epigenetic crosstalk in macrophage activation: an updated view

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Macrophages are innate immune cells that fulfill a broad range of functions in host defense, tissue homeostasis and repair, pathology and development. To perform these activities, macrophages show a high functional heterogeneity and adopt their phenotype in response to environmental cues. As such, these myeloid cells mostly keep us healthy, but their perturbed activation can also drive disease progression as observed in cancer and atherosclerosis. Therefore, macrophage activation is tightly controlled at different levels. About a decade ago, epigenetic mechanisms started to emerge as key regulators of macrophage activation. Almost in parallel to this epigenetics revolution, the booming field of immunometabolism research revealed that distinct macrophage activation cues show different metabolic profiles that are tailored to support the macrophage's functional specialization. The molecular mechanisms that explain how metabolic rewiring drives macrophage function are now being revealed. In an earlier special report in *Epigenomics*, we proposed that "epigenetics could serve as a bridge between altered macrophage metabolism, macrophage activation and disease" [1]. This statement was highly hypothetical since both the macrophage epigenetics and metabolism research fields were still very much in their infancies. At that time, macrophage epigenetics reports were mostly centered around only two histone-modifying enzymes; HDAC3 and JMJD3, also known as KDM6B. HDAC3 promotes inflammatory (so-called M1) macrophage activation and represses anti-inflammatory (M2) cues in macrophages, while JMJD3 supports distinct macrophage activation modes [2,3]. Currently, a large quantity of histone-modifying enzyme has been linked to macrophage activation in one way or the other [4].

In parallel, the (macrophage) immunometabolism field in 2015 still was very unsophisticated with glycolysis being stigmatized as pro-inflammatory and mitochondrial fatty acid oxidation (FAO) as anti-inflammatory [5–8]. We now know that this view is too simplified since increased glucose utilization is also required for anti-inflammatory macrophage activation [9,10], and FAO can also drive inflammatory responses [11]. In fact, recent research with new genetic models challenges the common dogma that FAO supports anti-inflammatory macrophages [12,13]. These new findings highlight that care is needed when interpreting experiments that were performed using pharmacological inhibitors as they often show side effects [14]. Overall, research in the last few years revealed how intracellular metabolic changes in immune cells not only fuel energy production and biosynthesis, but also drive cellular phenotypes and functions. The molecular mechanisms by which metabolic rewiring is translated into altered gene expression and effector functions remained relatively scarce and are now being revealed. In this context, it became clear that metabolic enzymes and metabolites can have distinct non-metabolic effects that regulate gene expression and effector functions in macrophages. As an example, the glycolytic enzyme glyceraldehyde-3-phosphate can bind inflammatory mRNAs such as TNF to regulate their translation [15]. Moreover, itaconate

emerged as a key ‘immunometabolite’ that accumulates specifically in inflammatory macrophages and serves as a kind of anti-inflammatory safeguard via distinct mechanisms [16].

We previously hypothesized that epigenetic enzymes could serve as interpreters between cellular metabolism and macrophage phenotype [1]. This statement was based on studies in nonimmune cells demonstrating that the activity of epigenetic enzymes is regulated by the availability of the metabolites they use as co-factors or substrates. How did our hypothesis stand the test of time?

In the last couple of years, it became apparent that the Krebs cycle intermediates acetyl-CoA, α -ketoglutarate (α -KG), succinate and fumarate affect the epigenetic machinery and landscape of macrophages and thereby regulate gene expression and function. While this knowledge is still relatively scarce, it is now clear that metabolic–epigenetic crosstalk does indeed regulate macrophage activation.

Concerning acetyl-CoA, Covarrubias and colleagues were the first to discover that IL-4 induces Akt/mTORC1 signaling to activate ACLY [17]. This key metabolic enzyme converts citrate into acetyl-CoA, which can induce epigenetic remodeling through altered histone acetylation. Activation of ACLY is associated with increased intracellular acetyl-CoA levels, enhanced histone acetylation and expression of *M2* genes. Accordingly, pharmacological inhibition of ACLY suppresses a subset of *M2* genes, at least in mouse macrophages. It is worth noting that the role of ACLY in regulating IL-4 macrophage responses is currently under debate since a recent study reported that silencing ACLY in human macrophages did not affect the expression of a selected set of *M2* genes [18]. Additionally, this human study revealed that the ACLY inhibitors still affected IL-4-responses in the absence of their target protein. As such, the field awaits the validation of earlier observations with ACLY inhibitors using new genetic mouse models.

In parallel to acetyl-CoA, α -KG was demonstrated to accumulate in IL-4-induced macrophages through increased glutaminolysis [19]. α -KG regulates the activity of α -KG-dependent JMJD histone demethylases and ten-eleven-translocation family enzymes that control DNA demethylation. In macrophages, the IL-4-induced production of α -KG was shown to be particularly important for JMJD3-dependent epigenetic remodeling and the associated induction of *M2* genes. While α -KG acts as a co-stimulatory metabolite for JMJD3, the structurally related succinate rather inhibits this enzyme. As such, the α -KG/succinate ratio modulates the macrophage’s inflammatory status via JMJD3-mediated epigenetic rewiring. Increased α -KG abundance and a high α -KG/succinate ratio promotes anti-inflammatory *M2* activation, while a low ratio promotes pro-inflammatory *M1* responses. Artificial modulation by α -KG supplementation tweaks the balance in favor of *M2* activation and protects mice against septic shock.

Fumarate is another Krebs cycle intermediate that is structurally related to α -KG and induces epigenetic changes through inhibition of α -KG-dependent histone demethylases. By doing so, fumarate accumulation integrates metabolic and epigenetic programs in trained innate immunity [20]. This emerging concept refers to the observation that innate immune cells show a kind of memory after they are triggered, and this enables them to respond stronger to a secondary stimulus. Epigenetic rewiring is an integral part of this ‘training’ program since the deposited histone marks can be sustained for days or weeks, even after the initial trigger that induced them has vanished.

Overall, cross-fertilization between the epigenetics and immunometabolism research fields clearly revealed that metabolic–epigenetic crosstalk indeed regulates macrophage phenotypes as we and others postulated earlier. Yet, the lessons we learned over the last few years are just the tip of the iceberg and key questions still need to be resolved.

A key point to be addressed is how Krebs cycle intermediates like acetyl-CoA and α -KG elicit specific epigenetic, transcriptional and functional outcomes. Some enzymes and genes appear to be more affected by metabolite availability than others. Several hypotheses are raised which could explain the specificity of metabolite reactivity. Possibly, the effect of particular metabolites on the epigenetic landscape and resulting macrophage function is cell- and context-dependent and could be determined by the intrinsic chromatin structure. Conceivably, the regulatory regions of some genes need more remodeling to initiate transcription than others. Another way to induce specificity is the subcellular localization of metabolites and the plausible co-localization of enzymes that produce and consume them. Could such complexes of metabolic enzymes and histone-modifying enzymes act as a kind of assembly line to produce high amounts of metabolites at the right place and time? Identifying the location of metabolite pools within the cell and understanding how this compartmentalization changes over time will aid in the quest to gain further insight into metabolic–epigenetic crosstalk in cells. While experimentally challenging, this spatiotemporal view should shed light on the fundamental mechanisms by which metabolic changes are translated into alterations in gene expression, macrophage function and ultimately disease outcome. Before we can even think about translating this knowledge to the clinic, we need to consider the fact that studies in human cells and *in vivo* data are currently very limited. We have clearly entered exciting times in which key questions need to be resolved. Back to work!

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References

1. Baardman J, Licht I, de Winther MP, Van den Bossche J. Metabolic–epigenetic crosstalk in macrophage activation. *Epigenomics* 7(7), 1155–1164 (2015).
2. Hoeksema MA, Gijbels MJ, Van den Bossche J *et al.* Targeting macrophage histone deacetylase 3 stabilizes atherosclerotic lesions. *EMBO Mol. Med.* 6(9), 1124–1132 (2014).
3. Van den Bossche J, Neele AE, Hoeksema MA, de Winther MP. Macrophage polarization: the epigenetic point of view. *Curr. Opin. Lipidol.* 25(5), 367–373 (2014).
4. Rodriguez RM, Suarez-Alvarez B, Lopez-Larrea C. Therapeutic epigenetic reprogramming of trained immunity in myeloid cells. *Trends Immunol.* 40(1), 66–80 (2019).
5. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol.* 38(6), 395–406 (2017).
6. Vats D, Mukundan L, Odegaard JI *et al.* Oxidative metabolism and PGC-1 β attenuate macrophage-mediated inflammation. *Cell Metab.* 4(1), 13–24 (2006).
7. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* 38(4), 633–643 (2013).
8. Huang SC, Everts B, Ivanova Y *et al.* Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat. Immunol.* 15(9), 846–855 (2014).
9. Huang SC, Smith AM, Everts B *et al.* Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity* 45(4), 817–830 (2016).
10. Van den Bossche J, Baardman J, Otto NA *et al.* Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Rep.* 17(3), 684–696 (2016).
11. Moon JS, Nakahira K, Chung KP *et al.* NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages. *Nat. Med.* 22(9), 1002–1012 (2016).
12. Divakaruni AS, Hsieh WY, Minarrieta L *et al.* Etomoxir inhibits macrophage polarization by disrupting CoA homeostasis. *Cell Metab.* 28(3), 490–503 (2018).
13. Nomura M, Liu J, Rovira II *et al.* Fatty acid oxidation in macrophage polarization. *Nat. Immunol.* 17(3), 216–217 (2016).
14. Van den Bossche J, Van der Windt GJW. Fatty acid oxidation in macrophages and T cells: time for reassessment? *Cell Metab.* 28(4), 538–540 (2018).
15. Galvan-Pena S, Carroll RG, Newman C *et al.* Malonylation of GAPDH is an inflammatory signal in macrophages. *Nat. Commun.* 10(1), 338 (2019).
16. O'Neill LAJ, Artyomov MN. Itaconate: the poster child of metabolic reprogramming in macrophage function. *Nat. Rev. Immunol.* (Epub ahead of print) (2019).
17. Covarrubias AJ, Aksoylar HI, Yu J *et al.* Akt-mTORC1 signaling regulates ACLY to integrate metabolic input to control of macrophage activation. *Elife* 5, pii: e11612 (2016).
18. Namgaladze D, Zukunft S, Schnutgen F *et al.* Polarization of human macrophages by interleukin-4 does not require ATP-citrate lyase. *Front. Immunol.* 9, 2858 (2018).
19. Liu PS, Wang H, Li X *et al.* Alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat. Immunol.* 18(9), 985–994 (2017).
20. Arts RJ, Novakovic B, Ter Horst R *et al.* Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab.* 24(6), 807–819 (2016).