composition of lipid droplets and microglial function will uncover the novel role of lipid metabolism in neuroinflammation and aging.

Overall, the exciting findings described in Marschallinger and colleagues provide new insights into microglial lipid accumulation in the aged brain and the role of lipid metabolism in microglial functions. However, the study also raises questions for future investigations. What are the differences between lipid-accumulating microglia and LDAM? Why is LDAM specifically observed in the hippocampus? Are LDAM present in other types of neurodegenerative diseases beyond Alzheimer's disease and, if so, do they play similar roles? Lastly, do metabolic changes contribute to the formation of lipid droplets or do lipid droplets mediate metabolic changes in microglia? Although these questions remain to be answered, the study by Marschallinger et al. opens the door to a better understanding of various microglia statuses that underlie neuroinflammation and brain homeostasis.

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Why Warburg Works: Lactate Controls Immune Evasion through GPR81

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Lactate accumulation in tumors—a hallmark of the Warburg effect—has recently been shown to regulate cancer cell metabolism and survival through autocrine activation of GPR81. Now, Brown et al. (2020) demonstrate that lactate surprisingly also controls immune evasion through paracrine activation of GPR81 on stromal dendritic cells.

A century ago, Otto Warburg described the phenomenon that cancer cells are highly glycolytic and convert the resulting pyruvate to lactate, which accumulates in large amounts (Warburg and Minami, 1923). This is a key element of the so-called "Warburg effect." In recent years, it has become evident that lactate is not just a waste product in tumors but is in fact highly important for cancer cell survival and growth. Recently, this effect was shown to be mediated at least in part through its ability to activate the lactate sensor, GPR81, a receptor that is highly expressed in cancer cells (Roland et al., 2014; Brown and Ganapathy, 2020). Thus, autocrine activation of GPR81 by lactate plays a key role in reprogramming the cancer cell metabolism to adapt to the special, harsh microenvironment of solid tumors and for upregulation of a series of general cancer cell defense mechanisms. Now Brown and coworkers report that GPR81 not only in cancer cells but also in stromal cells of the tumors is pivotal for tumor growth, and that, specifically, GPR81 expressed in antigen presenting dendritic cells may be key to cancer cell immune evasion (Brown et al., 2020).

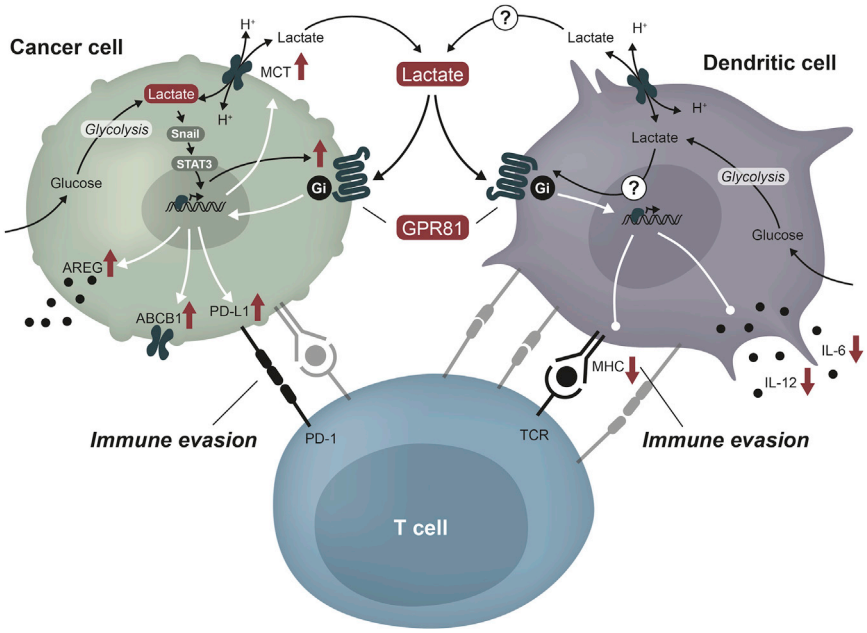

Figure 1. Overview of GPR81 Expression and Function in Cancer Cells and Immune Cells with Focus on Immune Evasion

Interaction between a cancer cell, an antigen-presenting dendritic cell, and a T cell. In the cancer cell, increased glycolysis will generate high concentrations of lactate, which is excreted to the tumor microenvironment through monocarboxylate transporters (MCTs), which co-transport protons (H^+). In cancer cells as opposed to the normal cells from which they originate, lactate increases expression of GPR81, at least in part through the transcription factor Snail and STAT3. White arrows indicate that autocrine stimulation of GPR81 by lactate, via Gi, upregulates a number of cancer cell survival mechanisms including MCTs (lactate transport), amphiregulin (AREG) associated with angiogenesis, ABCB1-mediated efflux of xenobiotics (chemotherapeutics), and inhibitory checkpoint ligands like PD-L1 involved in immune evasion at the cancer cell level. In the dendritic cell, GPR81 is also upregulated—through as yet unclear mechanisms—and its stimulation by lactate leads to decreased secretion of proinflammatory IL-6 and IL-12, downregulation of MHC, and consequently decreased tumor antigen presentation and activation of T cells, which all are associated with immune evasion at the immune cell level. As discussed in the text, the acidic microenvironment will likely favor inward transport of lactate, meaning that lactate may in fact accumulate mainly intracellularly in cancer cells and not in the interstitial tumor microenvironment as generally assumed. This will still allow for local autocrine stimulation of GPR81 but will not be compatible with a paracrine stimulation of lactate from cancer cells to immune cells. As indicated, it is however possible that in the tumor microenvironment lactate from the glycolytic dendritic cells themselves could instead stimulate GPR81 in an autocrine manner. The possibility that high intracellular lactate could also be involved in the upregulation of GPR81 in the dendritic cells in analogy with the cancer cells is also indicated.

GPR81 expression is normally restricted to adipocytes and relatively few other cell types of the body (Husted et al., 2017). However, GPR81 is upregulated with strikingly high incidence in a variety of solid tumors and cancer cell lines, including breast- and pancreatic cancers (Roland et al., 2014; Lee et al., 2016). Through shRNA knock-down, Roland and coworkers demonstrated that GPR81 expression is required for cancer cell survival in medium mimicking the tumor microenvironment, conceivably at least partly due to GPR81's involvement in the control of expression of monocarboxylate transporters (MCTs – i.e., lactate-proton co-transporters). Importantly, they showed that GPR81 is essential for xenograft tumor growth and metastasis *in vivo* in immunocompromised mice (Roland et al., 2014). Subsequently, other groups have shown that knock-down of GPR81 in cancer cells decreases their expression of pro-angiogenic amphiregulin and decreases angiogenesis *in vivo* (Lee et al., 2016), increases cancer cell susceptibility to chemotherapy (Wagner et al., 2017), and reverses the upregulation of inhibitory checkpoint ligands such as PD-L1 important for cancer cell immune evasion (Feng et al., 2017). Thus, through autocrine activation of GPR81, lactate drives a large number of cancer cell survival mechanisms (Figure 1).

Brown and coworkers now demonstrate that the lactate receptor is not only important for cancer cell survival and growth through its expression in the cancer cells themselves, but apparently plays a major role through its expression also in stromal cells (Brown et al., 2020). This was initially shown in PyMT transgenic female mice, who normally develop mammary tumors and lung metastases spontaneously. However, when crossed into *Gpr81*$^{-/-}$ mice, they display increased latency in tumor development and, in particular, strongly reduced ability to form metastases. Most convincingly, in an orthotopic, syngeneic cancer model, tumor growth of GPR81-negative AT-3 breast cancer cells was almost totally prevented in *Gpr81*$^{-/-}$ mice (Brown et al., 2020). This strongly indicates that GPR81 expression in stromal cells is required for tumor growth. Furthermore, profiling and RNA-seq analysis of tumors showed that the reduced tumor growth in *GPR81*$^{-/-}$ mice was associated with increased numbers of infiltrating T cells and dendritic cells and an increased immune surveillance profile. Accordingly, *in vitro* differentiated dendritic cells express GPR81, and both lactate and the non-metabolite GPR81 agonist CHBA suppressed cell surface presentation of MHC-II and suppressed spontaneous and TLR receptor-induced secretion of the pro-inflammatory cytokines IL-6 and IL-12. This strongly suggests that through paracrine activation of GPR81, tumor-derived lactate prevents dendritic stromal cells from presenting cancer cell-specific antigens to other immune cells (Brown et al., 2020) (Figure 1). As autocrine activation of GPR81 in cancer cells themselves also limits their recognition by T cells through upregulating checkpoint ligands such as PD-L1 (Feng et al., 2017), GPR81 is now positioned as a key mechanism of lactate-driven immune evasion in cancer.

However, many details remain unclear. First, although GPR81 is not normally expressed in, for example, healthy mammary glands and exocrine pancreatic and bronchial tissue, it is highly expressed in multiple different types of solid tumors derived from these and other tissues where GPR81 normally is not expressed (Roland et al., 2014). What drives this marked, tumor-specific upregulation? Very recently, Xie and coworkers discovered that GPR81 expression in lung cancer cells can be driven

by lactate itself through a GPR81-independent pathway involving upregulation of the transcription factor Snail, complex formation with STAT3, and STAT3 binding to the GPR81 promoter (Xie et al., 2020). Intuitively, it would make sense if the cancer cell-specific expression of GPR81, which clearly is crucial to cell survival, is driven by components of the tumor microenvironment, such as lactate itself. A similar mechanism could perhaps also be responsible for the high expression of GPR81 in stromal cells? It is also unknown how other characteristic features of the tumor microenvironment, such as acidic extracellular pH or hypoxia (Boedtkjer and Pedersen, 2020) impact GPR81 expression. In fact, we still need to learn in which stromal cell populations GPR81 is upregulated and which of these are key to the roles of the receptor in tumor growth and metastasis, as Brown and coworkers performed RNA-seq analysis of whole tumors and did not study isolated cell types (Brown et al., 2020). It is likely that GPR81 is upregulated also in, for example, tumor associated macrophages, fibroblasts and adipocytes and could play a role also in the ability of these cells to support tumor survival in the harsh tumor microenvironment (Boedtkjer and Pedersen, 2020). Importantly, the signal transduction mechanisms employed by GPR81 downstream of its canonical Gi signaling to reprogram cancer cells and deactivate immune cells is also still rather unclear. Interestingly, recent work identified a role for the co-transcriptional activator TAZ in GPR81-dependent PD-L1 upregulation in cancer cells (Feng et al., 2017), however, there are likely many other mechanisms yet to be discovered.

A bona fide paracrine function requires that lactate concentrations really are elevated in the tumor microenvironment as generally assumed. However, Rabinowitz and coworkers in a recent review pointed out that available data generally reflects total tumor lactate and that there is very little hard evidence for lactate concentrations being much higher in bulk tumor interstitial fluid than in circulation. They conclude that lactate accumulates mainly inside cancer cells and propose that this is probably due to the highly acidic extracellular environment in tumors, which favors outside-in transport by the lactate-proton MCT co-transporters (García-Cañaveras et al., 2019). In such a scenario, autocrine activation of GPR81 in the cancer cell is still a possibility whereas paracrine activation of GPR81 on more distant immune cells is more unlikely. However, since for example dendritic cell often display a strongly glycolytic metabolism and hence lactate production, GPR81 activation in these cells could well be autocrine instead of paracrine (Figure 1).

It is well established that high tumor lactate is associated with poor patient survival (Brizel et al., 2001). Interestingly, analysis of TCGA data now indicates that high expression of GPR81 is also associated with poor outcome in breast cancer patients (Brown et al., 2020). However, this effect is not independent of cancer stage and patient age, and further analyses detailing the relationship between GPR81 expression and outcome in patients are needed. The fact that GPR81 signaling is important for tumor growth and metastasis not only through its expression in the cancer cells but also in the immune cells makes the receptor a very interesting potential target to both prevent cancer cell survival and to restore the ability of the immune system to recognize and eliminate cancer cells. No GPR81 antagonists are today available in the public domain. However, GPCRs are in general good drug targets, and structure-based discovery of small synthetic non-metabolite ligands for metabolite receptors is in rapid development due to the many new GPCR structures being solved (Lückmann et al., 2020). So, probing of GPR81 as a new anticancer target should soon be possible.

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