An acid trip activates protumoral macrophages to promote hepatocellular carcinoma malignancy

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Tumor-associated macrophages (TAMs) promote metastasis and tumor cell extravasation, survival, and growth. In hepatocellular carcinoma (HCC), the presence of TAM subpopulations correlates with poor outcome. In this issue of the JCI, Ning et al. report on their use of cell culture, mouse models, and human data sets to investigate the interactions between aerobic glycolysis and carbonic anhydrase XII (CA12) expression in HCC. Aerobic glycolysis promoted CA12 upregulation in TAMs, which induced a protumoral phenotype to promote tumor growth and metastasis. Tumor cell factors derived from HCC samples induced CA12 upregulation in tumor-infiltrating TAMs via the HIF1α pathway. In preclinical models of HCC, CA12 inhibition reduced tumor growth and lung metastasis and reduced TAM infiltrate. Notably, dual treatment with anti-PD1 and CA12 inhibitors synergistically attenuated tumor growth and metastasis and enhanced survival compared with either treatment alone. These findings suggest that targeting CA12 in combination with immune-checkpoint blockade may provide treatment options for HCC.

Tumor-associated macrophages have protumoral functions

Tumor-associated macrophages (TAMs) are among the most abundant immune cells in the primary and metastatic tumor microenvironments (TMEs). In most cases in human cancers, their density is correlated with poor clinical outcomes (1). Data from mouse models in a wide range of cancers have shown protumoral activities of these cells. In the primary site, TAMs enhance tumor invasion and intravasation, initiate angiogenesis, and nurture stem cells, while in the metastatic site, TAMs promote tumor cell extravasation, survival, and persistent growth (2). Importantly, in both sites, macrophages suppress immune response from cytotoxic T cells and NK cells (3, 4). Such immunosuppressive activities, if translated to humans, have the potential to impair current immunotherapies (5). In mice, TAMs are very diverse, with each protumoral function driven by identifiable subpopulations (6, 7). However, some TAMs are also likely to be antitumoral, although their activities are overwhelmed by the greater numbers of protumoral ones. Similar diversity is found in human cancers (8).

In this issue of the JCI, Ning et al. focus upon hepatocellular carcinoma (HCC), which has a very high frequency with unfavorable outcomes in Southern China. Structurally this cancer has a stereotypic morphology in which subpopulations of TAMs associate with pathological features of the tumor (9). The research group has previously shown that some TAM subpopulations correlate with poor outcome, while other populations associate with good prognosis (10). These data suggest that targeting and altering the protumor population phenotype could provide therapeutic options in immunotherapy.

Warburg first described the concept that cancer cells exhibit an alternative metabolic state to permit proliferation and compensate for the inefficiency of energy production (11). This process is a fundamental switch in metabolism that replaces cellular respiration with high rates of aerobic glycolysis, which allows high glucose uptake even in the presence of abundant oxygen, producing lactic acid instead of carbon dioxide as a by-product. The lactic acid accumulates within the TME, creating an acidic environment. Simultaneously, the TME also becomes progressively hypoxic, resulting from increased proliferation and poor vascular perfusion, and tumors develop necrotic areas. A hostile milieu results, particularly for immune cells in TME. Ning et al. identified an autocrine molecular pathway in HCC that involves carbonic anhydrase XII (CA12). CA12, induced in macrophages during aerobic glycolysis, promoted TAM survival within the acidic environment. CA12 also induced a protumoral phenotype to promote tumor growth and metastasis (Figure 1).

Induction of CA12 in HCC-associated macrophages

Glycolysis-induced CA12 is the pivotal mediator between the acidic TME and downstream protective and tumor-promoting effects. CA12 belongs to a family of 15 carbonic anhydrases that are transmembrane enzymes involved in mediating extracellular pH. CA12 was first identified as being overexpressed in renal carcinoma cells (12, 13) and has subsequently been implicated in tumorigenesis of multiple cancers. Together, hypoxia, the acidic environment, the induction of CA12, and a poor prognosis suggest a role of CA12 in mediating tumor progression through the maintenance of the extracellular pH (14).
As the HIF1α pathway is highly associated with both hypoxia and aerobic glycolysis, Ning and colleagues explored its role in glycolysis-induced CA12 expression. Indeed, while the tumor-derived secreted factors induced HIF1α in the monocytes, the inhibition of HIF1α via siHIF1α and echinomycin attenuated CA12 expression, demonstrating that activation of the HIF1α pathway provides a molecular link between the acidic environment and CA12 expression. It was noted that HIF1α inhibition could only partially abrogate glycolysis-induced CA12 expression, suggesting that other mechanisms are involved.

To investigate the interactions between aerobic glycolysis and CA12 expression, Ning et al. performed in vitro culture assays on CD14+ monocytes that were isolated from peripheral blood of healthy donors, then treated with HCC-derived supernatants. Tumor cell–derived factors induced CA12 upregulation within the tumor-infiltrating TAMs in human HCC samples. This effect was dependent on aerobic glycolysis observed in the HCC samples, as CA12 upregulation was attenuated by the addition of glycolysis inhibitors 2DG and 3PO to the in vitro assay (Figure 1).

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that additional mechanisms mediate CA12 expression. The HCC cells also induced an array of cytokines from the mono-
cytes, including TNF-α, IL-10, and IL-1β, in a glycolytic-dependent manner. These cytokines further promoted CA12 expres-
sion, which was attenuated by the addition of relevant cytokine inhibitors, and thus identify an important autocrine loop with-
in the TAMs that, together with the glycol-
ysis-dependent effects via HIF1α, leads to sustained CA12 expression.

CA12 protects TAMs in the acidic environment
The upregulation of CA12 provides a pro-
tective effect and promotes the survival of macrophages within the acidic TME by preventing macrophage apoptosis. In preclinical mouse models of HCC, CA12 inhibition reduced tumor growth and lung metastasis and correlated with reduced TAM infiltrate (Figure 1). Through the utilization of in vitro assays in acidic cul-
ture conditions to mimic the in situ acidic TME, Ning et al. confirmed that tumor-in-
duced CA12 promotes the survival of macrophages in acidic conditions, which is attenuated in the presence of the CA12 inhibitors. These protective effects were not observed after macrophage deple-
tion using three independent depletion meth-
ods, and the tumor-promoting roles were abrogated in vivo. These data confirmed the role of the TAM-expressed CA12 in promoting tumorigenesis and metastasis. The findings also confirm that the induc-
tion of CA12 plays a role in promoting TAM survival in the acidic environment.

Role of CA12 in promoting tumor progression
In addition to the protective effects on macrophage survival, CA12 was hypo-
thesized to exhibit protumorigenic effects due to the positive correlation between the abundance of CA12’ macrophages within the HCC samples and metastatic potential of tumor patients. Ning et al. demonstrat-
ed that CCL8 expression in TAMs is reg-
ulated by the hypoxic and acidic environ-
moment via CA12, thus identifying upstream metabolic regulators of CCL8 secretion within the TME. CCL8 was also upregu-
lated in tumor-associated monocytes in a CA12-dependent manner via sustained p38 signaling (Figure 1). The abundance of CCL8’ TAM infiltrate is similarly increased in human breast cancer patient samples and also correlates with poor survival (8).

Within the model of HCC, secreted CCL8 acts upon the tumor cells to induce the epithelial-to-mesenchymal transition (EMT), thus potentially facilitating metas-
tasis. CCL8 has previously been identified as a prometastatic migration factor in sev-
eral mouse models, including breast can-
cer (8, 16, 17), glioma (18), and melanoma (19). CCL8 also acts as a chemoattractant to monocytes that differentiate to TAMs, thus providing a positive feedback loop (8). Thus, Ning et al. and previous studies support a role for CCL8 in promoting tumor cell invasion that subsequently promotes metastasis. However, targeting of CCL8 was not investigated in the in vivo HCC model; thus, the possibility that CCL8 is the effector of the increased metastasis remains unresolved.

Conclusion
Through their utilization of a large data-
hase of HCC clinical samples, preclin-
ical models of HCC, and complementary in vitro assays, Ning et al. described a pathway involving the induction of CA12 expression in TAMs during hypoxia. This pathway provides a protective effect to TAMs in the acidic environment and induc-
es CCL8 expression to promote tumor cell migration and metastasis. While similar roles of CCL8 have been previously identi-
fied, Ning et al. reveal that this hypoxia-in-
duced pathway in TAMs involves CA12 to protect TAMs in the TME.

Since the autocrine loop within TAMs originate in tumor cells, demonstrated by the effects of tumor supernatants on the TAMs, further analysis of the molecular factors upstream of HIF1α pathway activa-
tion should also be identified. While the molecular factors that induce HIF1α and subsequently CA12 were not analyzed, previous studies implicate hyaluronan as a potential mediator of the TAM phe-
notype. Kuang et al. demonstrated that tumor-derived hyaluronan fragments, which induce an immunosuppressive TAM phenotype (20), were responsible for activation of the glycolytic pathways in TAMs and regulated PD-L1 expression (15). Similarly, CA12 is a transmembrane receptor; therefore, future investigations should identify potential ligands.

Due to the pivotal role of CA12 in both protective and tumor-promoting effects in TAMs, Ning et al. inhibited CA12 to treat HCC by disrupting the autocrine loop within TAMs, which is induced in the acidic environment. Therapeutic tar-
geting of CA12 not only prevented the protumorigenic effects of downstream CCL8, but also increased the vulnerabil-
ty of TAMs, making them susceptible to the acidic environment. Moreover, the authors demonstrated that a combina-
tion of the anti-PD1 antibody and CA12 inhibitor had a synergistic effect in attenu-
tuating tumor growth and metastasis and enhanced survival compared with either treatment alone. Increased CD8+ T cells were also observed in the HCC TME after CA12 inhibitor treatment. The synergis-
tic effect with anti-PD1 therapy suggests that CA12 may also exhibit an immuno-
suppressve effect and that combination therapy may further reinitiate an antitu-
mor response within the TME. Together, these data demonstrate clinical relevance for targeting of CA12 in combination with immune-checkpoint blockade to provide therapy against HCC.

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