Linking Cancer Metabolism and Innate Immunosurveillance: Modulation of γδ T Cell-mediated Tumor Cell Recognition by the Key Metabolic Regulator AMPK

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Abstract

There is accumulating evidence demonstrating that γδ T cells comprise an important arm of innate cancer immunosurveillance governed by intracellular accumulation of mevalonate pathway products. In contrast to optimized in vitro conditions, growing tumors in vivo are exposed to nutrient deprivation/hypoxia both activating the key energy metabolism regulator adenosine monophosphate activated protein kinase (AMPK). Upon activation, AMPK increase catabolic ATP-generating processes such as uptake and oxidation of glucose and fatty acids but inhibit ATP-consuming biosynthetic processes such as protein synthesis and cholesterol production (mevalonate pathway). This correlation prompted us to investigate the effects of AMPK activation (mimicked by the synthetic AMP-analogue 5-aminoimidazole-4-carboxamide riboside (=AICAR) or by the anti-diabetic drug metformin on tumor cell recognition by Vγ9Vδ2 T cells.

We found that Vγ9Vδ2 T cells exhibited significant decreased up-regulation of activation markers in response to AICAR or metformin pre-treated target tumor cell lines (Daudi, RPMI 8226 and the farnesylpyrophosphate synthase (FPPS)-knockdown cell line Raji AS22) as compared to untreated tumor cells. In addition, AMPK activation in tumor cells reduced the ability of Vγ9Vδ2 T cells to produce pro-inflammatory cytokines (IFN-γ, TNF-α). As determined by phospho-specific flow cytometry analysis, either AICAR or metformin treatment resulted in increased phosphorylation and therefore inactivation of the AMPK target enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, subsequently lowering intracellular mevalonate pathway products critical for activation of Vγ9Vδ2 T cells. Thus, this data demonstrates for the first time an impact of cancer metabolism on immune recognition facilitating tumor escape.

Keywords: Immunotherapy; innate immune system; T cells; cancer metabolism; immune escape

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Introduction

Some of the earliest studies of cancer have observed alterations in tumor cell metabolism. In contrast to normally differentiated cells, tumor cells maintain a higher glycolytic rate, metabolizing glucose into lactate even in the presence of oxygen rather than through mitochondrial oxidative phosphorylation [1], a phenomenon known as aerobic glycolysis or the ‘Warburg effect’. A definitive explanation for Warburg’s observation remains elusive, but recent studies demonstrated that several oncogenic signaling pathways implicated in cell proliferation and cell cycle control also regulate metabolic pathways. AMPK, a highly conserved Ser/Thr protein kinase first identified to be activated by accumulation of intracellular AMP levels [2], is a central metabolic “fuel” sensor and a potent mediator of increased cellular glucose uptake which is a biochemical hallmark of many tumors and already in clinical use for the visualization of cancer by \(^{18}\)F-deoxyglucose positron emission tomography (FDG-PET). Upon activation (phosphorylation at threonine-172 [3]) AMPK inhibits several energy-consuming biosynthetic processes, including protein synthesis, gluconeogenesis and de novo fatty acid synthesis, specifically the generation of mevalonate in the cholesterol synthesis pathway [4, 5]. In addition, there is growing evidence that AMPK functions as key regulator of the adaption responses to nutrient and/or oxygen deprivation in the tumor microenvironment. Expression of activated AMPK has been shown in tumor tissues in vivo, especially around hypoxic necrotic regions in the center of the tumor, and activation of AMPK as well as Hypoxia-inducible factor-1 (HIF-1) support the survival of tumor cells through enhancement of transcription of hypoxia-inducible genes (e.g. vascular endothelial growth factor (VEGF)) and protection from energy deprivation-induced apoptosis [6-9]. Therefore, adaption of tumor cells to nutrient and/or oxygen deprivation by AMPK activation is a common feature where tumor growth advances a critical size and resources become scarce.

\(\gamma\delta\) T cells are a quantitatively minor subset of cytotoxic T cells but are known to play a crucial role in cancer immunosurveillance. A number of studies in mice have pointed to the capacity of \(\gamma\delta\) T cells to regulate malignancy. Girardi and coworkers demonstrated that after malignancy was induced either by inoculation of carcinoma cells or by chemical carcinogenesis, TCR\(\gamma\delta\) mice developed increased numbers of tumor sites and increased tumor burden compared to wildtype mice and that lack of \(\gamma\delta\) T cells is not compensated by \(\alpha\beta\) T cells or NK cells [10]. In addition to the potential of \(\gamma\delta\) T cells to lyse transformed cells in an NKG2D-dependent manner [11], these cells have also been shown to produce IFN-\(\gamma\) early in tumor development that in turn may regulate the function of tumor-triggered \(\alpha\beta\) T cells [12]. Although the studies of \(\gamma\delta\) T cells in mouse models have increased the understanding of these cells in humans, there are a number of differences of \(\gamma\delta\) T cell receptor (TCR) repertoires between mice and humans.

\(\gamma\delta\) T cells, which represent the vast majority of circulating human \(\gamma\delta\) T cells (50-70 % of \(\gamma\delta\) T cells and 0.5-7 % of CD3\(^+\) T cells), are not found in mice, but have also been shown to recognize a broad range of tumor cells of hematopoietic and non-hematopoietic origin [13-17]. They display a strong MHC-unrestricted cytotoxic effector activity against various tumor cells, directly involving the \(\gamma\delta\) TCR, and produce various anti-tumor cytokines, including tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interferon-\(\gamma\) (IFN-\(\gamma\)) [16, 18, 19]. Metabolites that stimulate \(\gamma\delta\) T cells in a TCR-dependent manner have first been purified from microorganisms [20] and are phosphorylated non-peptide molecules (so-called phosphoantigens (PAgs)), which naturally occur in the microbial non-mevalonate
pathway of isoprenoid biosynthesis [21-24]. Recognition of tumor cells by human Vγ9Vδ2 T cells is linked to intracellular accumulation of endogenous mevalonate pathway (MVA) products such as isopentenyl pyrophosphate (IPP) or its adenine nucleotide derivate storage form 1-(adenosine-5'-yl) 3-(3-methylbut-3-enyl) triphosphoric diester (ApppI) [25]. Therefore, mevalonate metabolite reactive Vγ9Vδ2 T cells have the unique capacity to distinguish transformed cells from normal cells on the basis of recognizing a dysregulated cholesterol metabolism in cancer cells [15, 23, 25]. Vγ9Vδ2 T cells are also activated by several synthetic compounds that selectively stimulate Vγ9Vδ2 T cells either by mimicking natural PAgS (e.g. bromohydrinpyrophosphate, BrHPP) or by aminobisphosphonates (e.g. pamidronate or zoledronate), inducing intracellular accumulation of MVA products via inhibition of the IPP-consuming enzyme farnesylpyrophosphate synthase (FPPS) [17, 22, 26, 27]. These synthetic PAgS have already been used in clinical studies for selective in vivo stimulation of Vγ9Vδ2 T cells, where objective tumor responses could be observed, indicating the potential of human γδ T cells for cancer immunotherapy [28, 29]. Recently, studies identified a key role for Butyrophilin-3A (BTN3A), a member of the extended B7 receptor family, in PAg-mediated activation of γδ T cells. The mechanism is still unclear and is speculated to function either by presenting PAgS in a manner homologous to antigen presentation by MHC molecules or by binding PAgS to the BTN3A intracellular domain subsequently leading to recruitment of additional factors and/or rearrangement of its extracellular domain whereas additional genes located on chromosome 6 are essential for PAg-mediated γδ T cell activation [30-34].

In this study, we investigated the consequences of adaptive changes in cancer metabolism by AMPK activation on activation and effector functions of mevalonate metabolite reactive Vγ9Vδ2 T cells. We demonstrate that AMPK activation in tumor cells impairs tumor cell recognition and effector functions by tumor-reactive Vγ9Vδ2 T cells and may provide an alternate strategy for immune escape.

Materials and Methods

Reagents

Bromohydrinpyrophosphate = BrHPP (Innate Pharma, Marseilles, France), recombinant human interleukin IL-2 (Novartis, Basel, Switzerland), IFN-γ secretion assay detection kit (Miltenyi Biotec, Germany), human TNF-α and human IFN-γ ELISA kit (eBioscience, San Diego, USA), metformin (Merck, Germany), 5-aminoimidazole-4-carboxamide riboside (AICAR, Sigma-Aldrich, Germany), anti-phospho-AMPKα (Thr172) antibody, anti-beta-Actin (13E5) antibody (Cell signaling technologies (CST), USA), anti-phospho-HMG-CoA reductase (Ser872) (Millipore, Germany), anti-rabbit IgG-FITC (Jackson ImmunoResearch, Newmarket, UK), Phosflow Fix Buffer I (Becton Dickinson (BD), Germany)

Flow cytometric analysis

Cells were harvested after indicated culture periods and analyzed by two- or three-color flow cytometry (FACScan flow cytometer; BD) using the CellQuest software. Cells were stained with the appropriate concentrations of following mAbs: FITC-conjugated anti-pan γδ TCR, anti-Vδ2 TCR, anti-hULBP (R&D Systems, Abingdon, UK), anti-mouse IgG2a isotype control (BD, Germany), PE-conjugated anti-CD3, anti-CD69 (Beckman Coulter, Germany), anti-human MIC A/B, anti-mouse IgG2a isotype control (BD, Germany) and anti-IFN-γ (Miltenyi Biotec).
**Cell lines and cell culture**

$\gamma\delta$ T cell lines were established as described recently [35]. The human permanent cell lines Daudi (Burkitt lymphoma) and RPMI 8226 (multiple myeloma) were obtained from DSMZ (Germany). The Raji-FPPS knockdown cell line (Raji AS22) was described recently [35]. All cell lines were grown in RPMI 1640 medium (Gibco, Germany) supplemented with 10 % FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mmol/L L-glutamine (PAN Biotech).

**FACS staining of surface marker CD69**

For evaluation of CD69 expression, $1 \times 10^5$ target cells were seeded in 96-well micro titer plates, incubated with AICAR or metformin for 4 hours or overnight at indicated concentrations, washed twice with 1x HBSS (Gibco) and further co-cultured with $1 \times 10^5$ freshly prepared PBMCs from healthy donors. After 5 to 16 hours incubation, cells were harvested, stained with FITC-conjugated anti-$\gamma\delta$ TCR and PE-conjugated anti-CD69 and further analyzed by flow cytometry.

**IFN-$\gamma$ secretion assay**

$1 \times 10^5$ target cells, pre-treated with different amounts of either AICAR or metformin for 4 to 16 hours as indicated, were co-cultured 1:1 with effector cells in 96-well round-bottom micro titer plates. After overnight incubation, cells were harvested and analyzed for IFN-$\gamma$ secretion using the IFN-$\gamma$ secretion assay detection kit (Miltenyi Biotec) according to the manufacturer’s instructions.

**TNF-$\alpha$ and IFN-$\gamma$ ELISA**

To evaluate the effect of AICAR and metformin on the cytokine production of $\gamma\delta$ T cells either human TNF-$\alpha$ or IFN-$\gamma$ ELISA kit (eBioscience) were used according to the manufacturer’s instructions. $1 \times 10^5$ target cells were seeded in 96-well micro titer plates, incubated with AICAR or metformin for 4 hours or overnight at indicated concentrations, washed twice with 1x HBSS (Gibco) and further co-cultured with $1 \times 10^5$ PBMCs from healthy donors. After 5 to 16 hours incubation supernatants were collected and frozen at -20°C, till performing the cytokine assay.

**Intracellular staining of phospho-HMG-CoA reductase and phospho-AMPK**

For phospho-AMPK and phospho-HMG-CoA reductase analysis, Daudi cells ($5 \times 10^5$ cells/well) were kept in 200 µl medium either untreated or treated with 2mM AICAR or 10 mM metformin for 10-60 minutes, as indicated. Cells were harvested at indicated time-points and immediately fixed with an equal volume of Fix Buffer I (BD) for 10 minutes at 37°C. After centrifugation the pellets were resuspended in 90% ice-cold methanol and vigorously shaken for 30 minutes at 4°C. The cell suspensions were washed twice with PBS containing 3% BSA (PBS/B), resuspended in 100 µl anti-phospho-HMG-CoA reductase antibody solution (1:100 in PBS) or 100 µl anti-phospho-AMPKα antibody solution (1:100 in PBS) and incubated at room temperature for one hour. After two rinses with PBS/B, cells were incubated for 30 minutes with 100 µl of FITC-conjugated anti-rabbit IgG (1:100 in PBS) and further analyzed by flow cytometry. Intact cells were gated and the mean fluorescence intensity (MFI) was determined.
Western Blot analysis

For western blot analysis Daudi cells (5x10^5 cells/well) were kept in 200 µl medium either untreated or treated with 2 mM AICAR or 10 mM metformin for 30 minutes and after harvesting washed once with 1x PBS (Gibco). After centrifugation (5 min, 3000 rpm) cell pellets were resuspended in 100 µl 1x SDS-sample-buffer (62.5 mM Tris pH 6.8, 2% SDS, 10% glycerin, 50 mM DTT), sonicated 3 x 15 seconds on ice and heated 5 min at 95 °C prior to separation. Proteins were separated by 10% SDS-PAGE and transferred to a PVDF transfer membrane (GE Healthcare). Expression levels of phosphorylated AMPK were determined using anti-phospho-AMPKα antibody, as loading control β-Actin expression was detected.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). All experiments were repeated at least three times. Significance was calculated using Student’s t-test. P value less than 0.05 was considered statistically significant.

Results

Effect of AMPK activators on γδ T cell activation by tumor cells

To obtain an in vitro model, that would allow us to study the effects of AMPK activation in tumor cells on γδ T cell stimulation, we used two pharmacological compounds to activate AMPK. Published reports have demonstrated that antidiabetic drugs such as metformin or the more specific, cell-permeable adenosine analogue AICAR induce intracellular AMPK activation [36-38]. We therefore cultured two representative Vγ9Vδ2 T cell sensitive tumor cell lines (Burkitt’s lymphoma cell line Daudi or the multiple myeloma cell line RPMI 8226) in the presence of AICAR or metformin. Recognition of these tumor cell lines by Vγ9Vδ2 T cells has already been associated with intracellular accumulation of mevalonate metabolites or its adenine nucleotide derivatives such as IPP or ApppI, respectively [15, 25]. To investigate the capacity of AICAR or metformin treated target tumor cells on activation of primary γδ T cells, total PBMCs from healthy donors were co-cultured with these tumor cell lines and subsequently analyzed by two-color FACS staining for expression of the activation marker (CD69) on Vγ9Vδ2 T cells (Figure 1). To rule out direct negative effects of AMPK activating compounds on γδ T cells in our experimental settings, primary PBMCs were stimulated with the synthetic PAg BrHPP in presence of AICAR or metformin. As shown in Figure 1A, no statistically significant difference in up-regulation of CD69 was found between the PAg-induced positive control and experiments in presence of AICAR, thus excluding direct inhibitory effects of AMPK activators on γδ T cells. In addition, pre-incubation of tumor cells with AMPK activators followed by extensive washing, preserved the effects of AMPK activation on γδ T cell stimulation, so all further experiments were carried out after removing AICAR and metformin, respectively prior to co-culture with PBMCs. As shown in Figures 1A and 1B, AMPK activation in target tumor cells by either AICAR or metformin significantly reduced tumor cell-induced activation of Vγ9Vδ2 T cells. We observed a dose-dependent reduced induction of activation markers after treatment of Vγ9Vδ2 T cell sensitive tumor cell lines with AICAR or metformin. Inhibiting effects of AMPK activators on γδ T cell activation were confirmed in several healthy donors, although individual differences in CD69 expression levels were
observed. Thus, recognition of tumor cells by Vγ9Vδ2 T cells was faithfully suppressed by AMPK activation.

Figure 1 Effect of AMP-activated protein kinase (AMPK) activators on γδ T cell activation by tumor cells. Daudi cells, pre-incubated overnight with 2mM AICAR, were co-cultured with PBMCs after AICAR was removed by washing. As control PBMCs were stimulated with 1µM BrHPP, BrHPP (1µM)/AICAR (2mM) or medium alone for 16h and further analyzed for up-regulation of CD69. (A) Representative analysis of CD69 activated Vγ9Vδ2 T cells. (B) Daudi or RPMI 8226 tumor cells were incubated either with escalating doses of AICAR or metformin overnight as indicated. Then PBMCs from healthy donors were added (ratio 1:1) or PBMCs were incubated with BrHPP (1 µM) or medium alone, and after 16h incubation Vγ9Vδ2 T cells were analyzed for up-regulation of CD69. Each bar represents mean values ± standard deviation (SD) of triplicate cultures of one healthy donor. Student’s t-test was used to determine statistical significance of detected differences. *P≤ 0.05 was considered significant. Similar results were obtained from at least three different donors.

Modulation of γδ T cell effector functions by AMPK activation

An effector function of γδ T cells is the release of cytokines, particularly T helper 1 (TH1) cytokines Interferon-γ (IFN-γ) and Tumor necrosis factor-α (TNF-α), in response to target tumor cell lines [16, 18, 19]. To investigate the capability of AMPK activators to interfere with the secretion of cytokines on γδ T cells, target tumor cell lines were pre-treated with different amounts of either AICAR or metformin and after co-culture with PBMCs from healthy donors were further analyzed for intracellular cytokine production. Whereas a significant proportion of Vγ9Vδ2 T cells from the untreated control group secrete IFN-γ in response to target tumor cells, a significantly decreased fraction of the total Vγ9Vδ2 T cells produced IFN-γ in response to Daudi cells pre-treated either with
AICAR or metformin (Figure 2A). Figure 2B depicts a significant reduction of secreted IFN-γ in an ELISA after co-culture of PBMCs with AICAR pre-treated Daudi cells in comparison to either untreated tumor cells or PAg-stimulated PBMCs. Similar results were observed for TNF-α secretion, either for Daudi (Figure 3A) or RPMI 8226 cells (Figure 3B).

**Figure 2 AMPK activation modulates release of IFN-γ by γδ T cells.** Daudi cells were either kept untreated or pre-incubated with 2mM AICAR or 10 mM metformin for 4 h, co-cultured overnight with a BrHPP-induced VγVδT cell line or fresh PBMCs from healthy donors and further analyzed for IFN-γ production. (A) Representative FACS analysis of IFN-γ secretion of tumor cells co-cultured with a Vγ9Vδ2 T cell line from one donor. (B) IFN-γ ELISA of Daudi cells co-cultured with fresh PBMCs. Error bars indicate SD of triplicates stained for each sample. Data are representative of three healthy donors.

**Figure 3 AMPK activation modulates release of TNF-α from γδ T cells.** Either Daudi (A) or RPMI 8226 (B) tumor cells were incubated with 2mM AICAR or 10mM metformin for at least 4 h and further co-cultured overnight with BrHPP-induced Vγ9Vδ2 T cell lines. TNF-α levels were determined by ELISA in cell culture supernatants. Each bar represents mean values ± SD of triplicates from one representative healthy donor. Similar results were obtained from at least three different healthy donors.

**AMPK modulates γδ T cell-stimulated effector functions of Raji-FPPS knockdown cells**

Activation of AMPK leads to direct phosphorylation of various downstream enzymes, like HMG-CoA reductase, the key regulator of the cholesterol and mevalonate pathway [39]. HMG-CoA reductase activity is regulated by phosphorylation/dephosphorylation, whereas the unphosphorylated enzyme represents the active form of HMG-CoA reductase and the phosphorylated the inactive one [39]. To confirm that impaired tumor cell recognition and effector functions of γδ T cells are linked to reduced IPP levels as consequence of reduced HMG-CoA reductase activity, a recently described Raji-FPPS knockdown cell line (Raji AS22) was used. In this cell line the IPP-consuming enzyme FPPS of the
mevalonate pathway is inhibited due to short hairpin RNA (shRNA) and hereupon accumulation of IPP is sufficient to induce Vγ9Vδ2 T cell stimulatory activity in otherwise non-stimulatory Raji cells [35]. As shown in Figure 4, co-culture of PBMCs with Raji AS22, pretreated with AICAR or metformin, resulted either in decreased up-regulation of activation markers (CD69) on γδ T cells (Figure 4A) or reduced levels of secreted IFN-γ (Figure 4B). To rule out the possibility that NKG2D is involved in AMPK-dependent modulations of tumor recognition, the expression of the most common NKG2D-ligands MHC class I related chain A/B (MIC A/B) molecules and UL16 binding protein 1 (ULBP1) on the surface of the tumor cell lines after treatment with either AICAR or metformin was investigated. In our experiments no significant differences in the expression of NKG2D-ligands on tumor cells after AICAR/metformin treatment could be detected (Figure 5). Thus, our results rather argue for a dominant role of the Vγ9Vδ2-TCR in sensing changes in cancer cell metabolism induced by AMPK.

**Figure 4**

**Figure 5**

**Figure 4** AMPK modulates γδ T cell-stimulating effects of Raji-FPPS knockdown cells. Raji-FPPS knockdown cells were either incubated overnight with AICAR (2mM) or metformin (20mM) as indicated co-cultured for 16h with PBMCs from healthy donors and further analyzed for (A) expression of CD69 activation marker or (B) IFN-γ secretion. Error bars indicate SD for triplicates stained for each sample. Student’s t-test was used to determine statistical significance of detected differences. *P ≤ 0.05 was considered significant. Data are representative of three different healthy donors.

**Figure 5** Expression of NKG2D-ligands on the surface of tumor cell lines. The γδ T cell target tumor cell lines Daudi, Rajii AS22 and RPMI 8226 were either kept untreated or pre-incubated over night with 2mM AICAR or 10mM metformin, stained with (A) anti-huULBP1 or (B) anti-human MIC A/B and further analyzed by flow cytometry. Live cells were gated using forward sideward scatter plots and the mean fluorescence intensity (MFI) was determined. Data are representative of at least three independent experiments.
Enhanced phosphorylation of AMP-activated protein kinase and HMG-CoA reductase

To confirm that either AICAR or metformin are capable to activate AMPK and further phosphorylate HMG-CoA reductase and to determine differences in the relative levels of phospho-AMPK and phospho-HMG-CoA reductase intracellular antibody staining followed by flow cytometric analysis were performed. Treatment of Daudi cells with either AICAR or metformin resulted in detectable increases of phosphorylated and therefore activated AMPK and also of phosphorylated (and therefore inactivated) HMG-CoA reductase (Figure 6). A significant increase in the Mean Fluorescence Intensity (MFI) of phosphorylated AMPK occurred either in AICAR and metformin treated Daudi cells compared to untreated tumor cells (Figure 6A). Similarly the amount of phosphorylated HMG-CoA reductase in Daudi cells strongly increased after pre-treatment with AICAR or metformin, respectively (Figure 6B). Western blot analysis with Daudi cells, treated at the same conditions as mentioned above, further confirmed the effects of AICAR and metformin on target cells resulting in increased expression levels of phosphorylated AMPK (Figure 6C). These data also support the proposed modes of action of AMPK activation on the mevalonate pathway regulation.

Figure 6 AICAR and metformin increase the phosphorylation status of AMPK and HMG-CoA reductase. Daudi cells were incubated with either 2mM AICAR (20 min), 10mM metformin (60 min) or medium alone, stained with (A) anti-phospho-AMPK or (B) anti-phospho-HMG-CoA reductase and further analyzed by flow cytometry. Live cells were gated using forward and sideward scatter plots, and the median fluorescence intensity (MFI) was determined. Western blot analysis with Daudi cells treated at the same conditions showed increased expression levels of phosphorylated AMPK. Blots were probed with anti-beta Actin to verify equal loading (C). Data are representative of at least three independent experiments.
Figure 7 AMPK is a key regulator of energy metabolism. Cellular stress, such as nutrient deprivation or hypoxia, and many pharmacological AMPK activators, such as metformin, thiazolidinedione or AICAR, activate AMPK by increasing the cellular AMP:ATP ratio and consequently promote its phosphorylation. AMPK in turn phosphorylates a variety of substrates to inhibit anabolic processes like fatty acid synthesis, gluconeogenesis and 3-hydroxy-3-methylglutaryl-CoA reductase the key enzyme of the mevalonate pathway. Phosphorylation and therefore inactivation of HMG-CoA reductase is followed by decreased levels of IPP and subsequently impaired effector functions of γδ T cells.

Discussion

Despite the large number of studies dedicated to assessing the multiple connections between cancer metabolism and proliferation, none of these investigations have addressed the impact of cancer metabolism on immune recognition [5]. In this study, we demonstrate for the first time that tumor cells whose metabolism is altered by activation of AMPK are less sensitive to recognition by γδ T cells which have been shown to contribute to host antitumor responses (Figure 7). Within the cellular immunosurveillance network human Vγ9Vδ2 T cells have the unique capacity to distinguish transformed cells from normal cells on the basis of their specificity for metabolites of the mevalonate (MVA) pathway to cholesterol [15, 23, 25]. Endogenous or exogenous (e.g. aminobisphosphonates (ABP)- or shRNA- mediated FPPS inhibition) mediated up-regulation of metabolites of the MVA pathway in transformed cells account for the broad antitumor activity of Vγ9Vδ2 T cells against hematological and solid tumor cell lines in vitro [13-16, 40]. In contrast to the promising in vivo antitumor activities of Vγ9Vδ2 T cells against hematological malignancies derived from SCID mice models or first pivotal clinical trials in humans, several preclinical and clinical studies have shown only limited antitumor effects of mevalonate metabolite reactive Vγ9Vδ2 T cells in established solid
tumors [28, 29, 40-42]. Based on our observations, the continual supply of nutrients and oxygen under optimized in vitro or in vivo conditions for leukemic cells and most hematological malignancies affects tumor cell recognition by Vγ9Vδ2 T cells and might have contributed to the lower sensitivity of established solid tumors to Vγ9Vδ2 cell-based immunotherapy approaches. This hypothesis is further supported by the finding that in contrast to other innate lymphocyte subsets (NK cells, other γδ T cell subsets) mevalonate metabolite reactive Vγ9Vδ2 T cells show only minimal infiltration of established solid tumors in vivo [41, 43]. Moreover, density and location (center of tumor vs. invasive margin) of T cell infiltrates in solid tumors such as colorectal cancer have been shown to be of high prognostic value independent of classical TNM classification [44]. Therefore, our results suggest that cancer metabolism (e.g. hypoxic tumor centers) can influence the prognostically relevant location of tumor infiltrating lymphocytes and shape the local innate and adaptive immune responses.

The current concept that the innate and adaptive immune system can recognize and destroy nascent transformed cells encompasses a complex process (e.g. immunoediting) consisting of three phases: elimination, equilibrium and escape [45]. Data from mouse studies highlighted the role for γδ T cells in the elimination phase of cancer immunoediting [10, 46], whereas cancer immunosurveillance in humans and especially the role of human γδ T cells has been difficult to assess. To become clinically detectable in immunocompetent hosts, tumor cells must circumvent both innate and adaptive immunosurveillance. One of the best known mechanisms to evade naturally occurring or therapeutically induced adaptive T cell-mediated immune responses is loss or down-regulation of MHC class I molecules in tumor cells [45, 47]. Adaption of nascent tumors to nutrient and/or oxygen deprivation by AMPK activation during the equilibrium phase of the immunoediting process, leading to impaired recognition of mevalonate metabolite reactive Vγ9Vδ2 T cells, provides the developing tumor with an additional mechanism to escape immune detection. Thus, our observation supports other published data that a tumor may directly inhibit innate and adaptive antitumor immune responses by multiple mechanisms. Notably, these data presented here were obtained from in vitro studies of an experimental model with a limited number of tumor cell lines. Given the enormous diversity of neoplastic diseases and the complex interactions between metabolism, tumor growth and immunosurveillance, further studies are needed to extend our findings to other experimental and clinical tumor models. However, our findings provide a proof of concept that cancer metabolism can shape the cancer immunoediting process.

An important additional implication of our observations is that two classes of widely used diabetes drugs (biguanides such as metformin and thiazolidinediones such as rosiglitazone) which pharmacologically activate AMPK are able to abrogate activation of Vγ9Vδ2 T cells. Similar inhibitory effect on Vγ9Vδ2 T cell activation has been shown for other pharmaceutical upstream MVA inhibitors such as HMG-Co reductase inhibitors (statins) [15, 48]. Because oral antidiabetics such as metformin and cholesterol-lowering drugs such as statins are among the most widely prescribed drugs worldwide, concomitant use of these drugs may not by ideal for future γδ T cell-based immunotherapy approaches.

In summary, our findings not only introduce a potential additional mechanism of tumor-dependent innate immune escape but also have profound effects of our understanding and treatment of cancer since concomitant medications (e.g. metformin) as well as the type (leukemic vs. solid tumor) and stage of cancer (measurable tumor vs. minimal residual disease) are critical for the design of metabolic directed Vγ9Vδ2 T cell-based immunotherapy trials in the future.
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Abbreviations

AICAR: 5-aminoimidazole-4-carboxamide riboside
AMPK: adenosine monophosphate activated protein kinase
ApppI: 1-(adenosine-5’-yl) 3-(3-methylbut-3-enyl) triphosphoric diester
BrHPP: bromohydrinpyrophosphate
FPPS: farnesylpyrophosphate synthase
HMG-CoA: 3-hydroxy-3-methylglutaryl-Coenzyme A
IPP: isopentenyl pyrophosphate; PAg: phosphoantigen

References


