Cell Cycle 9:2, 211-212; January 15, 2010; © 2010 Landes Bioscience

AMPK

A metabolic checkpoint that regulates the growth of EGFR activated glioblastomas

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Cancer is a genetic disease. Mutated oncogenes and tumor suppressor proteins activate signaling networks that underlie many of the characteristic hallmarks of cancer.[1] Cancer is also a metabolic disease. Long before the identification of oncogenes, Otto Warburg showed that cancer cells consume large amounts of glucose relative to normal cells,[2] forming the basis for 18F-FDG PET imaging. Instead of complete oxidation of glucose, most of the glucose-derived carbon in cancer cells is secreted as lactate, even under aerobic conditions. "Aerobic glycolysis," despite yielding less ATP per glucose molecule, is sufficient to meet the energy demand of the rapidly dividing cancer cells while redirecting glycolytic intermediates towards anabolic processes, to meet the increased demand for biomass in rapidly proliferating cancer cells.[3] Thus, the Warburg effect may help cancer cells meet multiple metabolic needs at once in a cell autonomous fashion unconstrained by systemic regulation. Many of the oncogenes and tumor suppressor proteins commonly mutated in cancer appear to regulate these metabolic changes including increased glucose uptake, the switch to the Warburg effect,[3] and de novo lipogenesis.[4] A map of the connectivity between signal transduction pathways and metabolic networks is emerging that could potentially be therapeutically exploited.

The wiring map interconnecting signal transduction and cellular metabolism in cancer is in an early phase. Unexpected connections are likely to be uncovered. The B-RAF V600E mutation common in many types of cancer, including melanoma provides one such example.[5] This mutation promotes melanoma cell proliferation through Erk and Rsk signaling. Surprisingly, ERK and Rsk phosphorylate the LKB1 tumor suppressor protein, impairing its ability to activate the metabolic checkpoint protein AMP-activated protein kinase (AMPK),[6] an active suppressor of mTOR complex 1 signaling.[7,8] Thus, a metabolic checkpoint interconnects two commonly deregulated canonical signaling pathways providing a potential new therapeutic target.

Will the interconnections between signal transduction and cellular metabolism prove to be therapeutically exploitable? Recent work from our group uncovers an unexpected twist on the theme that suggests so.[9] Glioblastoma, the most common malignant primary brain tumor of adults contains EGFR amplification and mutations, including EGFRvIII, a constitutively active and ligand-independent mutant oncogene that strongly promotes PI3K signaling.[10] To date, EGFR tyrosine kinase inhibitors have been surprisingly ineffective in the clinic. Maintenance of signal flux through the PI3K/Akt/mTORC1 pathway, either as a consequence of PTEN loss, or via co-activation of other receptor tyrosine kinases (RTKs) is thought to be responsible,[11,12] although failure to inhibit EGFR-mediated changes in cellular metabolism has also been implicated in therapeutic resistance to EGFR inhibitors. Attempts to target EGFR/PI3K signaling downstream at mTOR complex 1 with rapamycin or its analogs have also failed in glioblastoma patients the clinic, in large part due to feedback activation of PI3K signaling.[13] Therefore, we set out to determine whether AMPK activation could block the growth of glioblastomas, and to assess whether EGFR activation regulated cellular response in vitro and in vivo.

The AMPK agonist AICAR strongly inhibited the growth of glioblastoma cells in vitro, with markedly enhanced efficacy in EGFR-activated tumor cells. Transfection of the constitutively active EGFRvIII allele sensitized tumor cells to AICAR. Consistent with this observation, AICAR was highly efficacious at blocking tumor cell growth across a panel of EGFR-activated cancer cell lines, even in cancer cell lines that were relatively insensitive to the mTORC1 inhibitor rapamycin. Similar results were obtained using a constitutively activated AMPK adenovirus and another AMPK pharmacological. The AMPK-specific inhibitor compound C and AMPK siRNAs inhibited these effects, demonstrating that they were mediated through AMPK. Thus, AMPK is a potent negative regulator of glioblastoma cell growth, particularly in EGFR-activated tumors.[9]

mTORC1 is thought to be a primary target of AMPK.[8] However, AICAR was more effective than rapamycin at blocking tumor cell growth despite being significantly less effective at suppressing downstream mTORC1 signaling. Reconstitution of PTEN diminished the anti-proliferative effect of rapamycin but not AICAR, suggesting that AMPK could potentially negatively regulating tumor growth through alternate pathways. AMPK phosphorylates a number of enzymes in addition to mTOR, including

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Submitted: 10/14/09; Revised: 10/26/09; Accepted: 11/06/09
Previously published online: www.landesbioscience.com/journals/cc/article/10540
Comments on: Guo, et al. Proc Natl Acad Sci USA 2009; 106:12932–7.

HMG-CoA reductase and Acetyl CoA carboxylase, pivotal enzymes in cholesterol and fatty acid synthesis, respectively. Unexpectedly, addition of the metabolic products of these enzymes, mevalonate and palmitate, largely rescued the inhibitory effect of AICAR on tumor cell proliferation. Thus, the anti-proliferative effect of AICAR on glioblastoma cell growth was mediated primarily through inhibition of cholesterol and fatty acid synthesis. Consistent with this, EGFRvIII expressing glioblastoma cells expressed significantly elevated levels of intracellular fatty acids, which were abrogated by AICAR treatment, and pharmacological inhibition of HMG-CoA and Acetyl CoA carboxylase mimicked the effect of AMPK activation on tumor cell growth. In vivo, AICAR potently and specifically inhibited the growth of EGFRvIII expressing glioblastomas. EGFRvIII significantly enhanced 18F-FDG uptake, as well as tumor growth rate, both of which were blocked by AICAR, suggesting that AMPK is a critical link between EGFR-mediated signal transduction and cellular metabolism.[9]

These results suggest a potential therapeutic strategy for targeting EGFR-activated gliomas and possibly other EGFR-activated cancers. As a practical challenge, AICAR and most of the other suite of current AMPK activating compounds (i.e., metformin) do not effectively cross the blood brain barrier. More brain accessible AMPK activating compounds need to be developed. Important mechanistic questions about the underlying molecular circuitry remain. In what ways does the molecular circuitry of a cancer cell differ from a normal highly proliferative cell such as a lymphocyte with regards to the interface of signal transduction and cellular metabolism and can salient therapeutic differences be identified and targeted? Developing a more refined map of the connectivity between signal transduction pathways and metabolic networks is likely to yield a much richer understanding of the biology of cancer, and is also likely to yield a set of potentially useful therapeutic targets. One thing is likely; surprises are in store.

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Acknowledgements

This work was supported by the National Institute for Neurological Disorders and Stroke (NS050151) and the National Cancer Institute (CA119347 and CA108633), and the Brain Tumor Funders' Collaborative. This work was also supported by the Harry Allgauer Foundation through The Doris R. Ullman Fund for Brain Tumor Research Technologies, the Henry E. Singleton Brain Tumor Program, and generous donations from the Ziering Family Foundation in memory of Sigi Ziering.

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