Cancer's sweet tooth: the Janus effect of glucose metabolism in tumorigenesis

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Lancet 2006; 367: 618-21

Department of Cardiology, Harefield Hospital, Harefield, Middlesex UB9 6JH, UK (H Ashrafian MRCP) ashrafian@hotmail.com Despite Otto Warburg's 1931 Nobel Prize for his work affirming the role of metabolism in carcinogenesis, there has been little further interest in this association between metabolism and cancer. Disinterest has, in part, been attributable to the notion that Warburg's description of a relation between a shift to glycolysis in carcinogenesis may be an epiphenomenon rather than a mechanistic determinant. By studying the critical cellular energy sensor AMP-activated protein kinase (AMPK), I postulate that the association between intermediary metabolism and tumours varies over time. Through accumulation of carbohydrates and pan-inhibition of AMPK, premalignant tumours may gain a replicative advantage through the repression of senescence. Conversely, malignant tumours, with a defective tumour suppressor contingent, undergo a "glycolytic switch", in part by tolerating a degree of AMPK activation, to mitigate substrate limitation. I contend that this Janus-faced relation with intermediary metabolism contributes to carcinogenesis; if proven, this finding would have important implications for public health, in that it would lend support to the idea that prevention of obesity, and caloric restriction and exercise could reduce the predisposition to cancer.

Clinical observation coupled with detailed pathological analysis, especially in hereditary models of colorectal tumorigenesis, have brought about the prevailing dogma that cancers arise by a stepwise evolutionary process.1 Increasingly sophisticated molecular techniques are allowing us insight into early molecular oncogenic stresses and their cellular counter-responses. By contrast, despite advances in the biology of tumour inflammation-immunology and angiogenesis, technical complexities have hampered progress in work on the intricate biochemical microenvironments of evolving tumours. For example, although we have known since the 1920s that advanced tumours have high rates of glycolysis,² we still know very little about the role of metabolic changes, including intermediary metabolism, that drive tumorigenesis. This topic is not only of interest for academic or treatment purposes, but also because the widespread use of 18F-fludeoxyglucose (FDG) PET-scanning in the assessment of tumours is based on the same principle-that upregulation of glucose metabolism is the result of an active selection process that confers a significant growth advantage on advanced tumours.

Here, through study of the biology of the energysensing enzyme AMP-activated protein kinase (AMPK) and exploration of the varying roles of glucose metabolism in early and advanced tumours, I draw attention to a paradox. That is, that adaptations that favour early selection of premalignant lesions may not necessarily concord (and may discord) with adaptations required for advanced carcinogenesis.³⁴

AMPK and intermediary metabolism

AMPK is an increasingly well characterised phylogenetically conserved critical enzyme. It is a heterotrimeric enzyme complex, composed of an α catalytic subunit and regulatory β and γ subunits. Because AMPK is exquisitely sensitive to, and is activated by, increased levels of 5'-AMP and inhibited by ATP and glycogen, it recognises and signals cellular energy levels.⁵ The enzyme associates closely with cellular glucose-glycogen and their metabolising enzymes through *KIS* domains within its β subunit. It is via this process that AMPK orchestrates intermediary metabolism.⁶

In broad terms, a fall in energy (ie, a drop in the ATP:AMP ratio) activates compensatory catabolism and inactivates anabolic pathways. Furthermore, a drop in cellular energy increases glucose transport into cells (through upregulation of GLUT1/4), glycolysis, and fatty acid metabolism. Processes that require energy, such as fatty acid synthesis, protein synthesis, and cell growth are switched off, through AMPK-dependent manipulation of mammalian target of rapamycin (mTOR), a central modulator of protein translation, cell division, and growth. An increased ATP:AMP ratio has the opposite effect.5 AMPK ultimately integrates signals at a cellular, systemic, and central (hypothalamic) level by elaborating and responding to adipokines (eg, leptin and adiponectin) to regulate food intake, bodyweight, and glucose-lipid homoeostasis.5

The recognition of yet another layer of AMPK control has come through the identification of AMPK's upstream activating kinases, *LKB1* and Ca(2+) calmodulindependent protein kinase, in various tissues.⁷ While confirming the crucial role that AMPK has in energy regulation, the identification of *LKB1* as the mutated gene in patients prone to cancer with the Peutz-Jegher's syndrome also provides a link between intermediary metabolism (via AMPK and other AMPK-related kinases) and carcinogenesis.⁸ How does AMPK contribute to the different stages of tumorigenesis?

AMPK and carcinogenesis

Much of our existing knowledge about the relation between metabolism and cancer pertains to advanced primary and metastatic cancers in human beings. There is evidence that upregulation of glycolysis per se may support carcinogenesis. Additionally, tumours, having undergone rounds of selection and proliferation, harbour defective mitochondria. Coupled later with an inadequate and inefficient angiogenic response, the evolving tumour is rendered hypoxic.9 To continue to generate adequate energy, nucleotides, phospholipids, and other raw materials for cell division-all of which are dependent on glucose provision-glucose uptake is upregulated. Transport of glucose into mammalian cells is limited by glucose transporter expression; accordingly, malignant cells have been found to overexpress GLUT1. Similarly, an upregulation of glycolysis in malignant tumours has been well recognised for over 80 years.¹⁰

It would seem rational, then, from a basic understanding of AMPK biology that AMPK should be activated to facilitate glucose metabolism and fatty acid metabolism in advanced cancers.11 However, as mentioned earlier, active AMPK also switches off anabolic processes, including proliferation through mTOR via the tumour suppressor tuberous sclerosis complex (TSC2).12 Active AMPK, therefore, acts to facilitate advanced tumorigenesis, but simultaneously reduces the selective advantage of cancer cells; this finding is supported by results from studies that suggest that AMPK activates the tumour suppressor p53 through its phosphorylation on serine 15 and leads to replicative senescence.13 The solution to this apparent AMPK paradox, at least for some advanced tumours, derives from the observation that advanced malignant cancers often retain a sufficient complement of defective downstream tumour suppressors, which allows a degree of AMPK activation, while mitigating the growth limiting effect of the enzyme. For example, the tumour suppressor p53 is mutated or dysfunctional in most cancers.14 Likewise, some cancers harbour TSC2 mutations, allowing AMPK activation without mTOR suppression.15

The situation differs for premalignant lesions; the less advanced tumour bulk with adequate blood supply permits early tumours to metabolise aerobically, generating ATP through high-turnover oxidative phosphorylation rather than low-turnover oxygen-sparing glycolysis. Moreover, no great selective advantage exists for premalignant cells that import excess glucose through AMPK activation since these cells have little substrate deficit. In fact, based on the observation that AMPK activation slows cellular proliferation, for premalignant cells that retain intact cellular senescence machinery such as p53, TCS2, and mTOR, active AMPK is a disadvantage.13 These cells should inhibit AMPK, an improbably arduous task for mutagenesis since there are many subunits to suppress. By contrast with more advanced lesions, premalignant lesions would be selected according to measures somatically instituted, to inhibit AMPK by increasing cellular ATP and glycogen and by reducing AMP. This end could be achieved through adipokine/LKB1 manipulation or by upregulation of glycolysis or fatty acid metabolism with increased mitochondrial activity and glycogen. To achieve this Figure: Tumorigenesis

AMPK repression the cellular set-point for accumulating carbohydrates and ATP could be reset higher, or AMPK subunits might vary with respect to their subcellular distributions. and their kinetic parameters. Correspondingly, while individual AMPK subtypes (eg, those with $\gamma 2/3$ subunits) might facilitate glucoseglycogen repletion, the bulk of cellular AMPK may be rendered quiescent. Furthermore, since the inhibition of senescence may be dependent on particular temporal and spatial parameters, AMPK inhibition may only be necessary at specific times and within isolated subcellular compartments.

The figure illustrates the sequence of events in tumorigenesis. As early well-organised normal cells undergo rounds of replication, taking the first steps towards tumorigenesis, they are adequately provisioned by substrate and oxygen. They do not need AMPK activity, and indeed suppress it with excess glycogen to attain a selective advantage that is essential at this stage of development. As these cells mutate further and escape tumour suppression, their increasingly uncontrolled growth leads to architectural heterogeneity within the tumour mass, accompanied by a failure of adequate perfusion despite exuberant angiogenesis. Hypoxic tumour cells express HIF1a and upregulate glycolysis through activation of AMPK. At this stage, the ability of AMPK to curtail cell growth is partially mitigated by the absence of effective p53 and other downstream tumour suppressors (TSC2) that heretofore have been decommissioned. Active AMPK in the malignant stage facilitates the "glycolytic switch" and provides a substrate-dependent growth advantage to malignant tumours.



Testing the hypothesis

I propose that early premalignant lesions benefit from a general inhibition of AMPK activity; conversely, more advanced neoplasms making the transition to malignancy will increasingly depend on a degree of AMPK activation to increase substrate availability and increase malignancy-favouring glycolysis. Accordingly, differing tumour stages will have diametrically opposite requirements with respect to intermediary metabolism and AMPK function.

This hypothesis could be tested in various ways. Experimental and clinical tumours of varying stages could be assessed for glycogen levels, GLUT transporters, FDG-PET, AMPK, p53, TSC2, and mTOR activity (with specific subunit assessment at the mRNA/protein level). Although results of preliminary studies concord with my hypothesis by supporting the existence of a glycolytic switch,10 with early tumours expressing more glycogen and less glucose metabolism than advanced ones,16-19 detailed prospective expression, and proteomic and metabolic profiling should be used to assess this hypothesis.²⁰ I note the logistical difficulty of identifying early tumours. There is, however, a wealth of experimental evidence that premalignant tumours are subject to senescence; late tumours are not.4 By use of similar cancer-prone animal models (eg. those expressing K-rasV12), early and late tumours can be prospectively studied.

A provocative approach would be to artificially increase and deplete cellular glycogen levels to assess the incidence of premalignant lesions and their progression. The morphology, glycogen levels, and clinical outcomes with the ensuing advanced tumours would be informative. Insights have been afforded by patients with inherited glycogen storage defects, who have excess liver glycogen and are predisposed to hepatic adenomata.²¹ Another approach might favour ectopic transgenic upregulation of AMPK subunits (eg, PRKAG2/3) in specific tissues to increase glycogen and assess cancer risk. AMPK upregulation is known to cause glycogen accumulation and hypertrophy in cardiac tissue;22 this could be artificially simulated in other tissues. The effects of this glycogen overexpression could be amplified in crosses with cancer-prone patients mentioned previously. The incidence and morphology of tumours and proliferation-senescence patterns can be assessed.

Clinicians are often presented with relatively advanced tumours. According to my hypothesis, these tumours would already have defunctioned control elements downstream of AMPK (eg, p53, TCS2); they may, therefore, express AMPK with impunity. One approach to both testing this hypothesis and identifying a clinical in-vivo therapeutic node would be to assess the effect of replacing p53 genetically with or without the addition of 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR; a potentially safe AMPK agonist). Clearly, p53 replacement should be successful on its own. Not only has this replacement been feasible in the laboratory, but it has also been shown to be safe. Successful replacement of lost p53 function has been achieved clinically by the use of recombinant adenoviral vectors in non-small cell lung cancer in combination with systemic chemotherapy.²¹ I predict that AICAR should enhance this effect on tumours, thus paving the way for the development of better AMPK agonists.¹²

We now appreciate that obesity and carbohydrate excess predispose people to cancer; accordingly, in cohorts born around World War II, caloric restriction has been shown to contribute to a lowering of colorectal cancer risk.24,25 Furthermore, animal studies have supported the notion that caloric restriction of an ad-libitum diet by 60% lowers the risk of cancer.²⁶ Perturbingly, the lack of a mechanistic rationale has, in part, curbed clinical and therapeutic interest in this important area of research. If correct, my hypothesis lends mechanistic support to the intuitively and commonly held notion that high carbohydrate intake contributes to the selective advantage of preneoplastic lesions, and dietary restriction (especially low carbohydrate diets) coupled with regular exercise might preferentially reduce the burden of early-and ultimately advanced-disease. The same effect might be achieved through AMPK activation by existing agonists like metformin, which may reduce cancer,27 or by the application of adiponectin analogues and other adipokines (such as leptin) that stimulate AMPK. This inhibition of tumorigenesis may also be accomplished by the use of novel endogenous peptide agents that have been shown to suppress appetite and, hence, facilitate weight loss.28 Clinical studies in this area are of great potential importance, since exogenous adipokines (or their elaboration through measures such as obesitypeptide therapy,28 or bariatric surgery) provide a link between adipocyte manipulation and prevention of systemic cancer through the adipokine-AMPK axis.

By contrast with existing arduous and expensive cancer treatments, interventions such as exercise and dietary restriction are relatively cheap, immediately practicable, and prophylactic. Despite the success of exercise and diet in ameliorating other medical conditions (such as diabetes and cardiovascular disease), there has not been universal uptake of these interventions. This lack of action could be because people seem to find it difficult to make changes to their lifestyle. Confirmation of this mechanistic link would hopefully contribute to a clear scientific rationale to support the development of an infrastructure to facilitate public health through lifestyle modification and to promote it unequivocally.

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