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News and Commentary

One carbon, many roads

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Changes in metabolism that contribute to cancer development have attracted much attention in recent years. Although many metabolic pathways have come under scrutiny, particular attention has been devoted to one-carbon metabolism, reactions that support the production of nucleotides for DNA replication, NAPDH for antioxidant defence and methyl groups for the modification of DNA, RNA and protein.[1] Targeting this pathway is not new; indeed, antifolates – which deplete the availability of one carbon carriers – were among the first drugs to successfully treat human malignancies.[2] Now, some 70 years on, this pathway is again under the spotlight as the source of new targets for cancer therapy.

A key component of the one-carbon pathways is the amino acid serine, which functions as a major source of one carbon units (Figure 1). Although cells can readily take up exogenous serine, enhanced activity of the *de novo* serine synthesis pathway (SSP), through which serine can be generated from glycolytic intermediates, has been shown to play a role in supporting the development of several cancer types.[3] The suggestion that inhibition of the SSP enzymes could show a selective therapeutic effect has led to efforts to identify markers of cancers that would be particularly vulnerable to such an approach.

Now a recent study from Kotakis *et al.*[4] has shown that concurrent loss of LKB1 and activation of KRas in mouse pancreas cells (a combination of events frequently seen in human cancers) leads to a strong upregulation of the SSP, accompanied by an acquired dependence of these cells on the pathway (Figure 1). Closer examination showed that this increase in serine synthesis was important to maintain the levels of S-adenosyl methionine, the methyl donor used for a wide variety of methylation reactions. In addition to the increase
in substrate availability for methylation reactions, the LKB1/KRas mutant cells also increased the expression of DNA methyltransferase enzymes, such as DNMT. Accordingly, these cells showed an increase in DNA methylation, which was dependent on the enhanced SSP activity. Interestingly, the metabolic and hypermethylation phenotype associated with the loss of function of LKB1 is, at least partially, mediated by the inactivation of AMPK, a key metabolic sensor that is downstream of LKB1 kinase activity.

Clearly, changes in DNA methylation could have a plethora of effects on cell growth and behaviour through the regulation of gene expression. Somewhat surprisingly, however, the location of methylation changes in the DNA of these cells did not correlate with genes that had altered expression, prompting the authors to look for other impacts of this modification. Intriguingly, the LKB1/KRas mutant cells showed significant enrichment of methylation at different retrotransposon repeats - repetitive sequences that are actively transcribed to control the expression of coding genes.[5] Methylation of these retrotransposons dampens their expression – and the authors were able to demonstrate that reintroduction of LKB1 or direct inhibition of the SSP resulted in less methylation and the recovery of expression of these elements. Finally, the LKB1/KRas mutant cells were shown to be selectively sensitive to depletion or inhibition of DNMT. The implication is that the double mutant cells become dependent on the methylation and dampening of expression of the retrotransposons, and so are vulnerable to interventions that lower DNA methylation levels. Importantly, this effect is not evident in cells carrying only LKB1 deletions or activated Kras. Accordingly, the therapeutic value of small-molecule DNMT inhibitors was greatly enhanced in tumours carrying both mutations, compared with those that retained LKB1.

The study raises a number of interesting questions and possibilities. Why LKB1 loss leads to the increased expression of SSP and one-carbon metabolism genes is not known, although the coordinate upregulation of this pathway has been described in other systems - for example, in response to Myc or Nrf2 activation.[6,7] Similarly unclear is why the expression of the methylases is also upregulated in these cells, although it is possible that this is through the same pathway as that controlling the serine synthesis enzymes. In common with many cancers with increased activity of the SSP, inhibition of this pathway (e.g., by using PHGDH inhibitors[8,9]) retards cell growth, even when exogenous serine is in plentiful supply. Again, why this should be the case is not clear, since all the downstream pathways involving one-carbon metabolism – including the methylation pathways highlighted in this study – can be supplied from serine that is not synthesised *de novo* but rather is taken up into the cell. Furthermore, other consequences of serine and one-carbon metabolism, such as nucleotide or NADPH production, were not altered by LKB1 loss. The authors suggest that context or tissue-related factors might influence the final outcome of activation of the SSP - although whether LKB1 loss will have the same result

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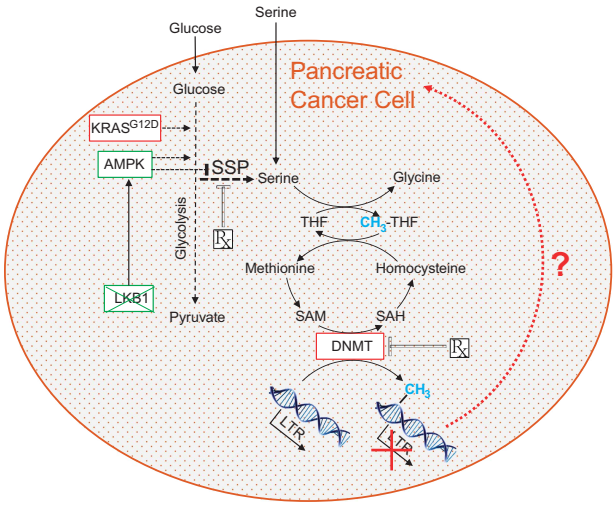

Figure 1 Metabolic and epigenetic changes associated with pancreatic cancer. KRAS oncogenic activation or LKB1 loss of function are known to control glucose metabolism, partly via AMPK. However, the co-occurrence of these oncogenic events in pancreatic cancer leads to increased *de novo* serine biosynthesis from glucose and to hyper-methylation and transcriptional silencing of retrotransposon elements. These events are required for tumorigenesis and hence they generate dependencies on serine biosynthesis and DNA methylation processes and, with that, potential stratified therapeutic strategies. Red or green boxes indicate proteins that are activated or inhibited, respectively. CH_3, methyl group; LTR, long terminal repeats (of retrotransposons); R_x, prospective prescribed therapeutic agents; SAH, S-adenosyl-homocysteine; SAM, S-adenosyl-methionine; SSP, serine biosynthesis pathway; THF, tetrahydrofolate

in other tumour types remains to be established. Another interesting but somewhat perplexing observation is the bias of methylation to retrotransposons in the KRas/LKB1 mutant cells. Why methylation of these regions of the genome is functionally important, while the methylation of nonrepetitive sequences (which was also altered in the double mutant cells) did not correlate with transcriptional regulation, will require some more investigation. Interestingly, a hypermethylation phenotype in intergenic regions was recently characterised in glioma carrying an oncogenic mutant form of isocitrate dehydrogenase.[10] In these tumours, the uncontrolled production of the oncometabolite 2-hydroxyglutarate leads to hypermethylation by the inhibition of DNA demethylases. However, in contrast to the current study, the intergenic regions in isocitrate dehydrogenase-mutated gliomas were not retrotransposons but rather insulators that separate enhancers from promoters and hence directly regulate gene expression, especially in coding gene-rich areas of the genome.

Whatever the mechanisms underlying the response to KRas and LKB1 mutation, one exciting possibility raised by this study is that tumours carrying this combination of alterations will be more sensitive to certain targeted therapies. Kottakis *et al.* focus on the response of these tumours to DNMT inhibitors, but equally these cancers could show selective sensitivity to inhibitors of SSP enzymes. Several PHGDH inhibitors have been described, and it would certainly be of interest to test them in this model system. Expansion of these studies into genetically engineered mouse models or even human trials will be most enlightening. Overall, there is a rapidly growing understanding of how some of the most commonly identified oncogenic genetic alterations impact on serine metabolism, with the hope that such knowledge will allow us to match new therapeutics targeting this pathway to the patients that are most likely to respond.

Conflict of Interest

The authors declare no conflict of interest.

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