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The KRAS-BCAA-BCAT2 axis in PDAC development

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Understanding the metabolic rewiring of pancreatic ductal adenocarcinoma is an emerging strategy to identify cancer-associated liabilities and improve treatment. A new study now elucidates the function of BCAT2 in the early stages of tumour development, providing insights that could stimulate novel therapeutic strategies.

The oncogenic mutations that drive cancer development induce the transformed cells to lock into a 'biosynthetic mode'. Metabolic adaptation is crucial for the tumour to meet its energy requirements and to produce the biomass necessary to sustain proliferation and survival[1]. In this rewired metabolic network, a central role is played by amino acids, which provide both carbon and nitrogen to ensure the production of macromolecules needed to enable tumour growth. *In vivo* studies on non-essential amino acid (NEAA) metabolism have shown that both oncogenic and tumour suppressor mutations can influence sensitivity to specific dietary NEAA starvation[2]. On the other hand, the metabolism of essential amino acids also plays a crucial role in cancer. In particular, the relevance of BCAA metabolism has become

evident in recent years[3, 4]. Specifically, either branched chain amino acids transaminases 1 or 2 (BCAT1, BCAT2) have been shown to be up-regulated and important for proliferation in neoplastic diseases such as glioma, acute myeloid leukaemia, non-small cell lung cancer and pancreatic ductal adenocarcinoma (PDAC)[3, 5-7]. The latter is one of the major cancer-related causes of death worldwide due to late diagnosis and chemotherapeutic resistance[8]. Despite the efforts to identify new drug targets the viable therapeutic options for PDAC remain limited. Previous studies have shown increased expression of BCAT2 in the late stage of PDAC[3, 7], however the potential benefit to the tumour of this upregulation remains unclear. In this issue of *Nature Cell Biology*, Li et al.[4] dissect for the first time the molecular mechanism by which the BCAA-BCAT2 axis is boosted in the early stages of Kras-driven PDAC, and how therapeutic BCAT2 inhibition or dietary BCAA restriction slow the onset of the disease.

In order to shed more light on PDAC metabolism, Li et al.[4] analysed amino acid concentrations in tumour and healthy tissue in a genetically engineered mouse model to evaluate the full progression of the disease, from pancreatic intraepithelial neoplastic (PanIN) lesions to invasive carcinoma (*LSL-KrasG12D/+; Pdx1-Cre* (*KC*)). While most of the non-essential amino acid levels were unchanged, the concentration of BCAAs was elevated in the tumour tissue. Leucine, valine and isoleucine are essential amino acids and can supply carbons and nitrogen to build other macromolecules in the cell[9]. The carbons provided by BCAA oxidation can be processed to acetyl-CoA, enter the tricarboxylic acid (TCA) cycle and be used in lipids synthesis[9]. Additionally, nitrogen derived from BCAAs can be utilised for nucleotide production[9]. Li et al. confirmed previous studies that observed the upregulation of BCAT2 in the advanced stage of PDAC[3], and demonstrated that levels of BCAT2, but not BCAT1 were significantly higher in the PDAC precursor PanIN lesions. To better validate the role of BCAT2 in the early stages of disease development, the authors took advantage of a KC mouse model in which *Bcat2* could be knocked out in an inducible fashion (KC/*Bcat2*$^{-/-}$). The *in vivo Bcat2* deletion not only attenuated the formation of PanIN

lesions, but also prevented their progression into a more advanced stage and mitigated the desmoplasia linked to the disease.

To further corroborate their findings and the relevance of BCAA catabolism in PDAC progression, Li et al.[4] demonstrated that *in vitro* downregulation of *BCAT2* decreased mitochondrial respiration and intracellular levels of Leucine-derived non-essential amino acids like Glutamate, Alanine, Serine and Aspartate, as well as nucleotides. In line with these results, the authors found a decrease in actively proliferating cells upon *in vivo Bcat2* knockout. Also, Li et al.[4] showed that survival in *KC* mouse models was significantly prolonged in orthotopic transplantation assay with either Bcat2 pharmacological inhibition or low-BCAA diet. While these data reveal information about the function of BCAT2, further research using labelled BCAA could trace their metabolic fate in PDAC development.

Next, the authors asked if the manipulation of *Bcat2* expression affected the concentration of BCAA-derived acetyl-CoA. Since a recent study showed that increased cytosolic acetyl-CoA levels were responsible for elevated histone acetylation resulting in acinar-to-ductal metaplasia and therefore in PDAC development[10], Li et al.[4] analysed acetyl-CoA pool and histone-lysine acetylation in both acinar and ductal cells. The authors showed that upon *Bcat2* knockout, acetyl-CoA metabolism and histone acetylation was indeed dramatically affected in acinar cells, but not in ductal cells. This reinforces their observations in which Bcat2 plays a central role during ductal-derived PanIN lesions by sustaining BCAA catabolism and thus promoting mitochondrial function and providing a nitrogen source.

To gain further insight into the molecular mechanism by which BCAA catabolism is up-regulated during PDAC development, Li et al. interrogated the effect of *KRAS* mutation on the expression of *BCAT2*. The authors analysed protein expression in clinical PDAC human specimens and observed a positive correlation between the increased levels of BCAT2 and *KRAS* mutation. Then, by manipulating *in vitro* the expression of *KRAS*, the authors demonstrated that BCAT2 regulation occurs at the protein rather than the

transcript level. In particular, using a tandem affinity purification and mass spectrometry approach, Li et al. demonstrated that ubiquitination of BCAT2 was operated by the E3 ligase tripartite motif-containing protein 21 (TRIM21), and that this process negatively correlates with *KRAS* expression. Next, the authors asked weather binding of TRIM21 was the result of a phosphorylation event on BCAT2. Li et al.[4] elegantly showed that spleen tyrosine kinase (SYK) directs the ubiquitination of BCAT2 by phosphorylation of tyrosine 228. Moreover, the authors also showed that the expression of SYK has a negative correlation, similar to TRIM21, with *KRAS*, thus explaining how the oncogene up-regulates BCAT2 at the post-translational level.

Finally, Li et al. focussed on the possible clinical relevance of their study by analysing their PDAC mouse model either during a low-BCAA diet or treated with long-term inhibition of Bcat2. KC mice fed a low BCAA diet displayed a reduction in the overall burden of the PanIN lesions. Notably, the low-BCAA diet impaired PDAC progression seemingly without affecting the global physiology of the other organs during the treatment. The only tissue displaying noticeable changes was the skeletal muscle, which showed fibres thinning. Li et al. showed that a regimen of 1/5 of BCAA in the food was enough to oppose PDAC-associated desmoplasia, impede PanIN progression and reduce the amount of actively dividing cancer cells.

This study highlights the great plasticity of tumour cells during tumour development. The authors pinpoint the critical role of the BCAA-Bcat2 axis at the onset of the disease and dissected the molecular mechanism explaining the upregulation of Bcat2 in the ductal cells that carry *Kras* mutation. Also, Li et al. suggest a possible therapeutic treatment based on a tailored dietary regimen. Proposing complementary diet together with pharmacological treatment against cancer is giving promising results in animal studies[2, 11]. A major challenge with dietary intervention will be to assess translatability to free-living human subjects. Furthermore, another interesting aspect identified in the treated animals by Li et al.[4] was an important reduction of the

desmoplastic stroma, which plays a fundamental role in both PDAC development and maintenance - further research could address this finding.

Figure Legend

Figure 1: KRAS mutation boosts BCAA metabolism promoting PanIN development. Acquisition of KRAS mutation by pancreatic ductal cells leads to the inhibition of SYK, resulting in reduced activity of TRIM21 and increased BCAT2 protein levels. Low-BCAA feeding diet or BCAT2 inhibition, impede PanIN progression.

Competing interests

ODKM contributed to CRUK Cancer Research Technology filing of UK Patent Application no. 1609441.9, and is a founder and shareholder of Faeth Therapeutics Inc. which is developing treatments for cancer.

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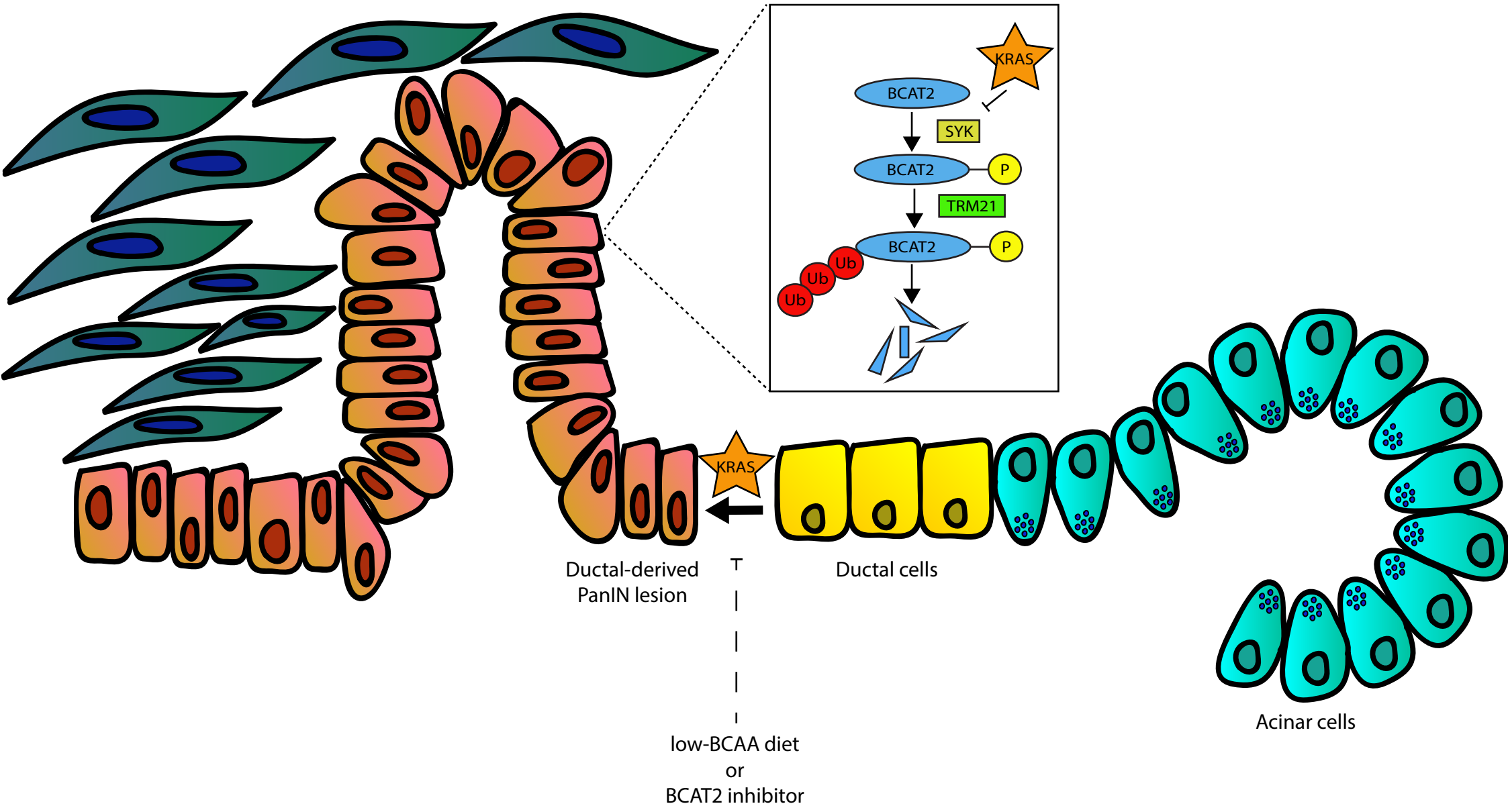
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