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Connecting LKB1 and AMPK links metabolism with cancer

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The tumor suppressor gene product LKB1 has been identified as the upstream activating kinase for the stress-responsive AMP-activated kinase, providing a link between regulators of cellular metabolism and cell proliferation in cancer.

Two researchers from the University of Dundee, Scotland - Grahame Hardie and Dario Alessi - have jointly solved a mystery that each had been puzzling over for several years (see 'The bottom line' box for a summary of their work). Hardie had been looking for the **upstream kinase** that regulates the metabolic regulator **AMP-activated kinase (AMPK)** (see the 'Background' box), while only a short walk away Alessi had been searching for the downstream target of the **tumor suppressor** and protein kinase **LKB1**. Like fitting two halves of a jigsaw together, the two researchers soon realized that each held the solution to the other's problem.

The bottom line

- AMP-activated protein kinase (AMPK) is activated in response to cellular stresses that deplete ATP, but its upstream activator kinase has proved difficult to purify and characterize.
- Searching for the upstream kinase(s) that regulate(s) the AMPK homolog Snf1 led to the discovery of Elm1 in yeast, and sequence comparisons revealed Elm1 to be a close relative of LKB1, a tumor suppressor gene product and protein kinase.
- Studies of LKB1 had shown its involvement in the cancer-prone Peutz-Jeghers syndrome, but its downstream mode of action was unknown.
- LKB1 has now been identified as the upstream regulator of AMPK, placing LKB1 - together with its binding partners STRAD and MO25 - in the middle of a crucial biochemical cascade that regulates cellular metabolism.
- This finding links the pathways controlling cellular metabolism and cell proliferation, and makes LKB1 an attractive potential therapeutic target for a wide range of clinical disorders.

Hardie has studied AMPK for well over a decade. He knew that it was switched on in situations of **metabolic stress**, such as low glucose or hypoxia, and by exercise in muscle, and that this required it to be phosphorylated by an upstream kinase. His team had tried purifying this upstream kinase activity and had attempted to determine the amino-acid sequence associated with the activity. But the upstream kinase is not an abundant protein and their approach was not proving successful.

In an attempt to speed things up, Hardie's group switched from human cells to brewers' yeast (*Saccharomyces cerevisiae*), an organism known to have a homologous kinase to AMPK called Snf1. With the help of two collaborators, Mike Stark in Dundee and Martin Schmidt in Pittsburgh, they could 'mine' the yeast genome sequence, which had been completed several years earlier, and use sophisticated analysis tools that are not yet as reliable for more complex mammalian systems. With this approach they

Background

- **AMP-activated protein kinase (AMPK)** is triggered in response to various forms of **metabolic stress** (such as exercise in muscle) that alter the relative concentrations of AMP and ATP within the cell.
- Having been activated by low ATP/high AMP, AMPK switches on catabolic pathways that generate ATP (such as glucose uptake and oxidation), while inhibiting ATP-consuming processes, such as biosynthesis and cell proliferation. AMPK thus plays a key role in regulating lipid and glucose metabolism and has been implicated in diabetes, obesity and cardiac diseases.
- Like many protein kinases, AMPK acts within a signaling cascade and is activated when it is phosphorylated by an **upstream kinase**.
- **Peutz-Jeghers syndrome** is a genetic condition that causes benign tumors and also puts people at greatly increased risk of developing more aggressive forms of cancer. **LKB1**, a protein kinase, is the product of the **tumor suppressor** gene *LKB1* which is mutated in cells from patients with Peutz-Jeghers syndrome.

identified Elm1, Pak1 and Tos3, three kinases that act upstream of Snf1 [1].

Hardie then returned to the human genome and searched for homologs of the three yeast kinases. The best match was a serine threonine kinase, STK11. "I'd never heard of it - it meant nothing to me," says Hardie. "But to my great surprise, when I looked it up on MEDLINE it turned out to be another name for LKB1, and I knew about LKB1 because Dario Alessi had been working on it, and he was just up the corridor."

For four years Alessi had been studying LKB1, and searching for its function. It had all the hallmarks of an important protein. Since its gene was sequenced in 1998, researchers had found 100 different mutations of this protein, all of which came from patients with **Peutz-Jeghers syndrome**, a genetic condition that puts people at risk of developing multiple benign tumors in the intestine as well as more aggressive forms of cancer. All the evidence suggested that LKB1 played a critical role in regulating cell proliferation.

"This wasn't the first time that Grahame Hardie and I had discussed a possible connection between LKB1 and AMPK," recounts Alessi. "Two years earlier we had thought about it and, although it was a very long shot at the time, had attempted a couple of experiments to see if my preparation of LKB1 would activate AMPK." But the experiments showed no sign of success and the idea was rapidly forgotten. Anyway, the coincidence that two people working in adjacent labs might be studying two protein kinases that also happened to be adjacent to each other in a biological pathway seemed too good to be true.

But this year, things were different. In the intervening time, Alessi's team (in collaboration with Hans Clevers' group in The Netherlands) had characterized LKB1 more closely and found that it was only active when joined to two subunits, Ste20-related adaptor protein (STRAD) and mouse protein 25 (MO25). The earlier activation attempts had been carried out using LKB1 alone. Hardie visited Alessi's lab late one afternoon, and by lunchtime the next day they had results from the first experiment. "We had hit the jackpot," claims Alessi, and the results are published in this issue of *Journal of Biology* [2].

Working together, they had combined the Alessi lab's human LKB1 and its subunits - which were expressed in cultures of human kidney cells (known as 293 cells, which readily take up DNA) - with AMPK that Hardie's group had made by expression of the rat protein in bacteria. In this reconstituted cell-free system they found that LKB1 massively activated AMPK. "The great thing was that because Dario had been working on LKB1 for three or four years, although without knowing what lay immediately downstream of it, we were able to make progress very quickly. He had all the right reagents and tools available to test our ideas," explains Hardie. Suddenly two areas of work joined together to form a whole that was much greater than the sum of its parts (see the 'Behind the scenes' box for more on the future of the work).

Some years earlier, Hardie's group had shown that AMPK needed to be phosphorylated by an upstream kinase at a site (Thr172) in the 'activation loop' of the kinase domain. It was also known that, when activated, AMPK had a negative influence on cell growth, and that AMPK is activated by various kinds of stress, particularly stresses that cause depletion of ATP. "It switches off any non-essential processes in the cell, one of which would be cell division," says Hardie. "If you are short of ATP you want to concentrate on surviving; you don't want to think about dividing."

On the other hand, LKB1 was known to be mutated in cells from patients with Peutz-Jegher's syndrome, and now there was the intriguing possibility that their disabled LKB1 leaves AMPK unable to perform its regulatory role, allowing cell proliferation to carry on unchecked. "This is a very important finding because it brings two

universes together, the small but important world of LKB1 and the well-researched world of AMPK," comments Jim Woodgett from Ontario Cancer Institute, in Toronto, Canada. "This is a new interface and in science, interfaces between areas are often scenes of rapid progress."

"It is a link between metabolism and cell proliferation, which is part of an emerging theme," agrees Tony Pawson, who works at the Samuel Lunenfeld Research Institute in Toronto. "In the rush of excitement with signal transduction over the past couple of decades there has been a tendency to think of metabolism as somehow boring, but it is now coming back with a vengeance, and it is exciting to see metabolism tie in so nicely to signal transduction."

Verification

More work was clearly needed to establish whether the early results were an artifact observed only in a reconstituted cell-free system, or whether the finding would also hold in living cells. The first step was to look at LKB1 and AMPK in HeLa cells. Alessi and his team already knew that LKB1 is not expressed in these highly abnormal transformed human cancer cells, and Hardie's team had previously discovered that HeLa cells produced AMPK, but that the kinase was not activated by treatments that usually activate AMPK. The answer seemed obvious - the lack of LKB1 explained the lack of activation of AMPK.

What's more, Alessi and colleagues had already introduced genes into a line of HeLa cells that restored their ability to produce LKB1. Now Hardie tested these cells and found that AMPK was indeed activated in them. It was the first evidence that LKB1 could activate AMPK in intact cells. "When we initially tried to publish this work, however, the reviewers expressed caution," notes Hardie. The issue was so big that they asked for a greater burden of proof. The problem was that HeLa cells, which are derived from a woman who died of cancer many years ago, have many other mutations as well as lacking LKB1. "The evidence showed that LKB1 was sufficient to activate AMPK, but because we could not rule out the possibility that other upstream kinases might also be missing in HeLa cells, we could not say that LKB1 was necessary," explains Hardie.

As a consequence, Hardie and Alessi approached Tomi Mäkelä at the University of Helsinki, Finland, who was growing immortalized mouse embryo fibroblasts in which the *LKB1* gene had been knocked out. "We were very fortunate that the Finnish group agreed to give us the cells so quickly," says Hardie. Soon, the Hardie and Alessi labs showed that AMPK was activated normally in wild-type mouse embryo fibroblasts, but in the cells from the LKB1 knockout embryos it was not activated. This time the evidence seemed compelling. "Now LKB1 was both sufficient and necessary," comments Hardie. "LKB1 is the upstream kinase in these cells, although we can't say that this is true in all cell types. There could be other upstream kinases in tissues like muscle, or elsewhere, but at least in mouse embryo fibroblasts LKB1 seems to account for all of the upstream kinase activity acting on AMPK."

The work also leaves open the possibility that LKB1 may influence many other downstream targets. "There are

Behind the scenes

Journal of Biology asked Dario Alessi and Grahame Hardie about the motivation for, and future of, their experiments linking LKB1 and AMPK.

What motivated this work?

"Both groups of researchers were driven by the desire to understand the control of metabolism and cell growth, and to see how this could relate to clinical diseases ranging from diabetes to cancer," agree Alessi and Hardie.

How long did the experiments take?

"I had been working on LKB1 for four years," says Alessi, while Hardie notes that he had been studying AMPK "for over a decade. Once the link was made, however, the work described in the current paper took a few weeks."

What are the next steps and what does the future hold?

"There are numerous questions to be answered," says Hardie. "For example, we currently know of 14 other human protein kinases that are closely related to AMPK but have no known functions. The question now is whether LKB1 activates these as well. If so, the tumor suppressor activity of LKB1 might be mediated through one of these as well as, or instead of, through AMPK. There is also a pressing need to discover the mechanism that enables phenformin to stimulate AMPK via LKB1. This might be the key to development of a second generation of phenformin-like drugs."

In addition, Alessi notes "there is still a lot of work to do to understand LKB1's interaction with STRAD and MO25, and how the combined unit performs its role in phosphorylating AMPK. STRAD itself appears to be a very interesting molecule in that its sequence suggests that it should also be a protein kinase, but it is an inactive 'pseudokinase'.

suggestions that LKB1 is controlling cell polarity," comments Pawson, adding, "one would imagine that this is through a different target - or is LKB1 only functioning as an AMPK kinase?" The degree of interest in this area is evident from the fact that while the Alessi and Hardie labs were carrying out these additional experiments, the groups of Marian Carlson and David Carling jointly published data pointing to the possibility that LKB1 might be the upstream kinase for AMPK [3].

Drugs, diabetes and cancer

At the same time as working on verifying LKB1 as the AMPK activating kinase, Hardie and Alessi had been testing their cells with two different drugs that stimulate AMPK in wild-type cells. One was phenformin, a close cousin of metformin, the active ingredient of the world's best-selling product for combating type II diabetes (Glucophage). In muscle, phenformin and metformin activate AMPK and thus mimic the effect of exercise, stimulating the cells to increase the uptake and metabolism of glucose and fatty acids. In cells that have no LKB1 - either HeLa cells or those from *LKB1* knockout mouse embryos - phenformin could not activate AMPK, but in modified HeLa cells containing the recombinant *LKB1* gene, or in wild-type mouse fibroblasts, the drug worked.

Alessi and Hardie found a similar situation with AICA riboside, another AMPK-activating drug that laboratory tests had shown could be used as a treatment for diabetes in animals. The molecule is converted inside the cell into AICA riboside monophosphate (ZMP), an analog of AMP. A few years earlier, Hardie had shown that the levels of AMP within a cell are critical indicators of cell stress, and are used by the cell as the signal that switches on AMPK to regulate metabolism. They now discovered that treatment of cells with AICA riboside also had no effect in the absence of LKB1.

With the number of people who have type II diabetes increasing dramatically around the world (it is estimated that there will be 200 million by the end of this decade), many pharmaceutical companies are looking for new ways of combating the disease. The patent has just lapsed on metformin, and in any case it is not a very potent AMPK activator, so there is a keen interest in developing a second-generation drug that will directly target AMPK. But it will not be easy. Most drugs are inhibitors, but what is required here is an activator. Now, however, there is a second option, because it may be possible to achieve the same results by targeting LKB1.

As well as having an impact on diabetes, the work has an obvious potential spin-off for patients with Peutz-Jeghers syndrome. "Pharmaceutical companies have been working with AMPK for years, so they must have numerous compounds on their shelves that they could screen for efficacy against the disease," says Woodgett. It may also be that studying LKB1 and AMPK holds the potential for yielding a new class of therapy for treatment of other more common types of cancer. One thing is certain - the two proteins are now hot property.

References

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