Biology

Targets and Mechanisms

Enigmatic caspase-2 unveiled

Chemotherapeutic drugs are often genotoxic agents that cause cancer cells to undergo apoptosis, or programmed cell death. The central components of apoptosis in human cells are members of the caspase protease family. Although caspase-2 was the second mammalian caspase identified about a decade ago, we still only have limited understanding in the mechanism of its activation.

Now, Tinel and Tschopp have given us a glimpse behind the mystery of caspase-2 regulation [1]. The unprocessed caspase-2 is a zymogen that contains a caspase-recruitment domain (CARD) that enables it to associate with RAIDD, a CARD and death domain (DD)-containing adaptor protein. Using recombinant protein technologies, immunoprecipitation and western immunoblotting, a complex containing caspase-2, RAIDD and PIDD (p53-induced protein with a DD) was identified in eukaryotic cells. This complex was named the PIDDosome and size-exclusion chromatography suggested that its molecular mass ranges from 550 to >670 kDa. The ablation of RAIDD and PIDD expression using small interfering RNA (siRNA) blocked caspase-2 processing, indicating that the PIDDosome is involved in the activation of caspase-2. Conversely, increased PIDD expression triggered caspase-2 activation and sensitized cancer cells to apoptotic cell death caused by genotoxins doxorubicin and etoposide. These results implicate the PIDDosome as an important component for mediating genotoxin-induced apoptosis.

By providing evidence for a caspase 2-containing PIDDosome, these authors have made a significant contribution to the field of apoptosis. However, investigators are still at the initial stages of dissecting the regulation of caspase-2 activity and the molecular pathways that this caspase is involved in. For example, it is possible that an endogenous inhibitor exists that modulates caspase-2 activity by regulating the formation of the PIDDosome, thereby affecting stress-induced apoptotic signaling pathways. As resistance to apoptosis is a hallmark of cancer, these unidentified molecules, as well as the identified components of the PIDDosome, could be of special interests as therapeutic targets for cancer treatment.

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1 Tinel, A. and Tschopp, J. (2004) The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science* 304, 843–846

The RNA-binding domain PAZ specifically recognizes the siRNA 3′ overhang

Short RNAs are processed into small interfering RNAs (siRNAs), which cause sequence-specific degradation of mRNA and gene silencing (known as RNA interference). PAZ is an RNA-binding domain that binds specifically to siRNAs. SiRNAs are 19–23-base duplexes with two-nucleotide 3′ overhangs, but it is not known how they are recognized. Ma *et al.* [2] have now solved the structure of a PAZ domain bound to a short RNA, showing that PAZ recognizes the 3′ overhang.

The structure of the PAZ domain of Argonaute was solved bound to a 9mer RNA, which formed an siRNA-like duplex with a two-nucleotide 3′ overhang at each end. A PAZ domain bound each overhang, forming a PAZ dimer. PAZ was also dimeric when bound to the RNA in solution, despite the individual PAZ molecules not contacting each other in the structure. Therefore, the PAZ domains bind independently to the 3′ overhangs, so a longer duplex probably binds in a similar manner.

Each PAZ monomer contacts the RNA strand containing the 3′ overhang along its entire length, but only contacts the 5′ terminal nucleotide of the other strand. There is a 110° bend at the end of the duplex, such that the 3′ overhang is inserted into a pocket on the protein containing several highly conserved residues. There are no specific contacts to the bases, suggesting that any combination of two nucleotides could be accommodated. However, there are specific hydrogen bonds to the 2′ OH of the ribose, explaining the reduced affinity for DNA. It will be interesting to see how other important determinants of siRNAs, such as the length, are recognized.

2 Ma, J.-B. *et al.* (2004) Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. (2004) . *Nature* 429, 318–322

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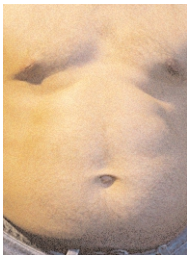
Liposuction without surgery



The past few years have seen an increasing awareness of the health risks that come with obesity. With only two, less-than-fully effective drugs available today, the potential market for anti-obesity drugs seems wide open for opportunities. Moreover, a number of hormonal and signaling pathways have recently been discovered that suggest where such drugs might intervene. In spite of this, finding successful drugs is not straightforward. Probably, evolution has endowed us with strong, redundant mechanisms to 'benefit' of rich, food supplies whenever – and no manner for how long – they become available. Fighting obesity has been compared with fighting cancer.

Remarkably, it is by using a therapeutic strategy borrowed from cancer research that a recent exciting breakthrough has been accomplished. In 2002, Wadih Arap (from the Texas M.D. Anderson University; http://www.mdanderson.org/) and co-workers had discovered that vascular endothelial cells in white fat tissue express a unique cell surface marker [3]. This marker had been discovered by probing different tissues and organs with complex libraries of peptide-expressing phages. The few phages that specifically bind can be grown in bacteria, and their sequence examined. The new work describes how the discovered 'fat-homing' peptide is indeed highly specific for vasculature that feeds white fat [4].

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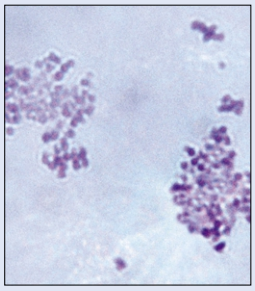
Microbiology

MBL-deficiency and *Staphylococcus aureus* susceptibility



Staphylococcus aureus is one of the major bacterial pathogens causing disease in humans with substantial morbidity and mortality. The defense against *S. aureus* includes humoral responses and phagocytic cells. In addition, some *in vitro* evidence has suggested a role for the mannose-binding lectin (MBL) complement pathway, rather than the classical or alternative pathways of complement activation.

To investigate the *in vivo* role of MBL, Shi *et al.* generated MBL-null mice and analyzed their susceptibility to *S. aureus* infection [5]. When infected intravenously, MBL-null mice had significantly increased mortality that could be partly restored to wild-type levels by treatment with human recombinant MBL (rhMBL). MBL-null mice had much higher levels of bacteria in blood, kidney, spleen and liver, and cytokine reponses (TNF-α and IL-6) were altered. Bacteria readily multiplied in blood from MBL-null mice, but were cleared from the wild type. Intraperitoneal injection of bacteria produced a significantly higher amount of abscesses, as well as higher bacterial loads in blood and organs in neutropenic MBL-null mice, when compared with neutropenic wild type mice. This phenotype could be reversed by rhMBL treatment of MBL-null mice. Furthermore, the ability of peritoneal macrophages to phagocytose bacteria was drastically reduced in MBL-null mice.

Taken together, these data clearly show that MBL and the MBL complement pathway are key players in the early response against infection by *S. aureus*, and possibly other bacterial pathogens. Furthermore, it suggests that MBL levels might be a marker of host resistance against infection that could be used clinically.

5 Shi, L. *et al.* (2004) Mannose-binding lectin-deficient mice are susceptible to infection with *Staphylococcus aureus*. *J. Exp. Med.* 199, 1379–1390

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In a further step, the investigators have linked this peptide with a second peptide that had earlier been shown to be taken up by cells, disrupt mitochondrial membranes and cause apoptosis (cell death). This apoptotic peptide is part of a strategy to cure cancer by starving metastases from their blood supply. When genetically obese mice were injected daily with 150 μg of the fat-homing/killing two-peptide construct, they lost a staggering 30% of their body weight over four weeks without any ill effects. Further analysis confirmed that this was due to loss of white fat following local vasculature apoptosis. Finally, the authors identified their 'target' cell surface protein as prohibitin, which is indeed restricted to white fat endothelium.

Given the significant risks that are associated with surgery to combat obesity (0.5–1% die from stomach reduction procedures), the prospects for this peptide as an anti-obesity drug look particularly good.

3 Arap, W. *et al.* (2002) Steps toward mapping the human vasculature by phage display. *Nat. Med.* 8, 121–127
4 Kolonin, M.G. *et al.* (2004) Reversal of obesity by targeted ablation of adipose tissue. *Nat. Med.* 10, 625–632

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