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Pot of gold for glioma therapy

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## Pot of gold for glioma therapy

**Cannabinoid agonists arrest tumor progression in a rodent model of malignant glioma. Will these molecules provide a starting point for new strategies for anti-cancer therapy (pages 313–319)?**

CANNABINOIDS HAVE HAD a long and interesting medical history. Soon after the British physician W. B. O'Shaughnessy had brought back from India an account of the remarkable effects of the cannabis plant, the medical communities in Europe and the US eagerly adopted it into their pharmacopeias. Cannabis, noted Robert Christison in his 1848 dispensatory, “promises to be an important article in *Materia Medica*...which deserves a more extensive enquiry than any hitherto instituted.”<sup>1</sup> Those propitious times would soon end, however, as a collective mood swing pushed cannabis and its medicinal properties into a limbo of scientific indifference.

And there it stayed for several decades—despite the lonely sounding of a few “voices crying in the wilderness”<sup>2,3</sup>—until about 10 years ago, when the serendipitous discovery of a brain receptor that binds cannabis-like compounds brought it back into the limelight<sup>4</sup>. The molecular cloning of the first cannabinoid receptor (now called CB<sub>1</sub>) was quickly followed by the identification of a second subtype in the immune system (CB<sub>2</sub>) and then by the characterization of two endogenous cannabis-like compounds with their attendant pathways of biosynthesis and inactivation<sup>5</sup>. These discoveries eventually led to the chemical syntheses of potent ligands (agonists and antagonists) selective for either receptor subtype, which have provided invaluable clues to help explain how the endogenous cannabinoid system may influence physiological functions as diverse as pain, movement control and blood pressure<sup>5</sup>.

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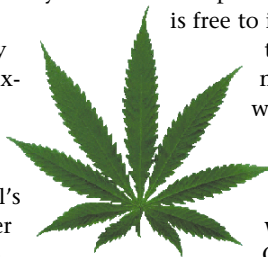
At the same time, these tools have rejuvenated Christison's plea for “a more extensive enquiry” into the medicinal potential of cannabinoids, allowing researchers to test popular notions such as their appetite-stimulating or pain-killing effects, as well as to explore newer avenues of research. In this issue of *Nature Medicine*, a paper by Galve-Roperh *et al.* provides an excellent example of the cannabinoids' therapeutic potential<sup>6</sup>.

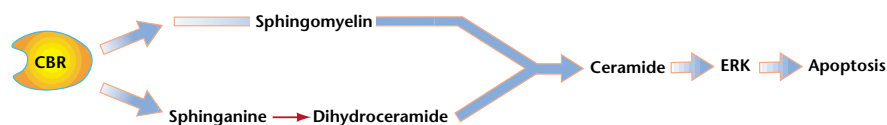
Galve-Roperh *et al.* report findings that indicate a new cannabinoid-based approach for the treatment of malignant gliomas. Malignant gliomas are a relatively uncommon but uniformly fatal form of brain tumor that can be modeled in rodents by inoculating glioma cells (for example, C6 cells) into the brain parenchyma. The resulting tumor grows very rapidly, leading to the animal's death within 2–3 weeks after the initial cell inoculation. Galve-Roperh *et al.* found that administration of cannabinoid agonists into the tumor by means of an osmotic pump connected to an intracerebral cannula eradicated the tumor in one-third of the inoculated animals, and prolonged the survival of another one-third for up to 6 weeks. In the remaining group of animals, the cancer was insensitive to the cannabinoids and continued its malignant course unhindered. Although incomplete, these findings must be

seriously considered, as glial tumors are peculiarly resistant to traditional therapy.

To identify which receptors are involved in the anti-cancer actions of the cannabinoids, Galve-Roperh *et al.* turned to a cell culture system. Based on their earlier observation that C6 glioma cells undergo programmed death (apoptosis) after exposure to a cannabinoid drug, they characterized this effect pharmacologically. Unexpectedly, they discovered that both CB<sub>1</sub> and CB<sub>2</sub> receptors are involved: CB<sub>1</sub> and CB<sub>2</sub> antagonists were able to prevent cannabinoid-induced cell death only if they were added together to the glioma cultures. This finding indicates that each cannabinoid receptor can trigger a full-fledged apoptotic response independently of the other, as long as it is free to interact with an agonist. Does this also occur *in vivo*? An affirmative answer to this question, which Galve-Roperh *et al.* did not address in their study, might be of considerable therapeutic importance. It would indicate that selective CB<sub>2</sub> receptor agonists can arrest the progression of malignant gliomas without exerting the psychotropic and hypotensive effects that accompany the recruitment of central and peripheral CB<sub>1</sub> receptors.

As with many other G<sub>i</sub>/G<sub>o</sub>-protein-linked receptors, agonist binding of CB<sub>1</sub> and CB<sub>2</sub> receptors causes inhibition of adenylyl cyclase activity and stimulation of mitogen-activated protein kinase activity<sup>7</sup>. However, the results reported by Galve-Roperh *et al.* do not support the





**Fig. 1** How cannabinoid receptors may induce apoptosis in C6 glioma cells. Occupation of either a CB<sub>1</sub> or CB<sub>2</sub> receptor (CBR) by agonist drugs may stimulate sphingomyelin hydrolysis and cause accumulation of ceramide. This reaction occurs within minutes of exposure to agonist, and may be catalyzed by the enzyme sphingomyelinase. Several days after the initial addition of cannabinoid agonist, induction of ceramide synthase activity may cause a more prolonged elevation of ceramide levels. Ceramide increases are in turn associated with activation of extracellular signal-regulated kinase (ERK) and the induction of apoptosis.

idea of direct involvement of these signaling pathways in the apoptotic actions of the cannabinoids. Rather, they indicate that cannabinoid-mediated glioma cell death may be signaled through accumulation of the lipid second messenger, ceramide, followed by activation of the extracellular signal-regulated kinase cascade (Fig. 1). Ceramide production is a ubiquitous cellular response triggered by cytokines, hormones and other intercellular mediators<sup>8</sup>. Galve-Roperh *et al.* found that cannabinoids cause a bi-phasic increase in ceramide levels in C6 glioma cells. The first phase of ceramide accumulation occurred within seconds or minutes after cannabinoid administration, which was likely to be a result of stimulus-dependent hydrolysis of sphingomyelin<sup>8</sup>. Two days after the addition of the drug, a second increase in ceramide levels took place, which coincided with the onset of the apoptotic response—probably reflecting an increase in *de novo* ceramide biosynthesis through the ceramide synthase pathway<sup>8</sup>. How these changes in intracellular ceramide intervene in apoptosis is unknown, but the fact that they are synchronized with increases in extracellular signal-regulated kinase and Raf-1 kinase indicates that these three factors may cooperate in mediating cannabinoid-induced glioma cell death.

But how likely is it that the discovery of anti-tumor effects of cannabinoids will affect malignant glioma therapy? At present, glioma patients who are subjected to an aggressive, multimodal treatment consisting of surgery, radiation therapy and chemotherapy have a median survival rate of 40–50 weeks<sup>9</sup>. This bleak scenario alone should provide sufficient motivation to continue the studies initiated by Galve-Roperh *et al.* The risk of typical cannabinoid side effects—euphoria, amnesia, decreased psychomotor performance and hy-

potension—may be outweighed by therapeutic advantages, and eventually be overcome through the development of selective CB<sub>2</sub>-selective agonists.

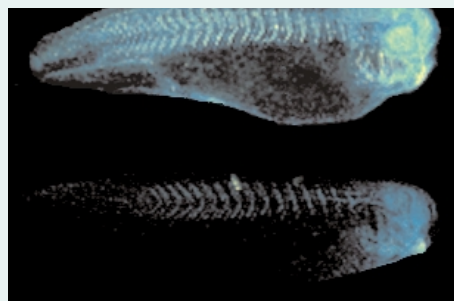
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## Visualizing transcription

Understanding the complex process of transcriptional regulation is essential for the study of normal development, and for learning how genetic mutations or exposure to teratogens can cause developmental defects. In the March issues of *Nature Biotechnology* and *Nature Medicine* (351), scientists have described ways to use magnetic resonance imaging (MRI) to glimpse gene expression as it occurs



deep within the interior of a living organism. Methods for visualizing domains of gene expression are not new to biologists—the current understanding of development has been shaped by studies in which gene promoters are fused to  $\beta$ -galactosidase ( $\beta$ -gal), or more recently, green fluorescent protein (GFP). However, because they depend on light microscopy, these techniques are limited in their ability to probe, with high-resolution, dense tissues that cannot be penetrated by light. In fact, according to Scott Fraser, one of the senior authors on the *Nature Biotechnology* paper, visualization of GFP expression with two-photon microscopy cannot be used to obtain images at a depth greater than 600  $\mu$ m. With the eventual goal of tracking gene expression domains in a living embryo dynamically over time, Louie *et al.* (*Nature Biotech.* **18**, 320–324, 2000) have developed a contrast agent called EgadMe (1-(2- $\beta$ -galactopyranosyloxy)propyl)-1,4,7,10-tetraazacyclododecane)gadolinium(III) that yields a robust MRI after being cleaved by  $\beta$ -gal. In the inactivated form EgadMe is unable to interact with water, making the molecule undetectable by proton MRI. However,  $\beta$ -gal enzymatically cleaves a sugar from the contrast agent, thereby freeing an interaction site for water and converting it to an active state that can be visualized by MRI. The picture shows two *Xenopus* embryos both injected with EgadMe at the two-cell stage. A few cell divisions later embryos were also injected with  $\beta$ -gal mRNA (top image) or left un-injected as controls (bottom image). When viewed by proton MRI, the cells injected with  $\beta$ -gal mRNA produce a high-intensity signal with 10  $\mu$ m resolution. Injection of cDNA encoding  $\beta$ -gal also resulted in a strong signal. With further development, this imaging technique will be useful in studying transcriptional regulation in a living mouse embryo *in utero*.

Natalie DeWitt