Do NSAIDs prevent colorectal cancer?

The author examines recent evidence from experimental and clinical studies

The idea that aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) might inhibit colorectal cancer began in the 1970s, when Bennett first noted that some human colorectal tumors overproduce prostaglandins compared with normal colonic mucosa[1,2]. Later studies confirmed that *in vitro* some (not all) large-bowel cancers do overproduce prostaglandin E_2 (PGE_2)[3]. Further, the concentration of PGE_2 in venous blood draining the tumors is higher, *in vivo*, when the cancers are large or locally invasive[4]. Most colorectal carcinomas and a subgroup of adenomas are known to overproduce prostaglandins by increasing expression of the inducible cyclooxygenase (COX)-2 gene, while maintaining background activity of the constitutively expressed COX-1 gene[5]. However, the central hypothesis continues to be Bennett's; certain colorectal tumors may self-promote by overproducing prostaglandins.

What we know

Results of animal experiments

More than 25 experimental studies in rodents confirm that high doses of several NSAIDs (indomethacin, piroxicam, sulindac, aspirin and others) inhibit chemically induced colon cancer in rats[6]. In the rodent model, NSAIDs reduce the incidence (percent of animals with tumors), multiplicity (number of tumors per animal) and size of chemically induced cancers. The model of chemically induced colon cancer in rats bears many similarities to human colorectal cancer, except for lower propensity to metastasize. Experimental proof in the rat does not equal certainty in humans, but does provide clues about the NSAID antitumor effect. Tumor inhibition occurs even when NSAIDs are first administered weeks after exposure to the chemical initiator and microscopic nodules are already present. The inhibition does not prevent cancer in all treated animals, and the tumors resume growth when NSAIDs are discontinued.

Results of clinical studies

Case studies and two small, randomized clinical trials show that the NSAID sulindac inhibits adenomatous polyps in patients with the rare hereditary familial adenomatous polyposis (FAP)[2]. Adenomatous polyps are the precursor lesion of most human colon cancer. People who inherit a mutated FAP gene and do not have their colons removed surgically develop numerous adenomatous polyps, and almost invariably by the age of 40 or 50 years they progress to colon cancer. Sulindac reduces the number and size of new polyps in patients with FAP and causes regression of existing polyps. However, as was found in the rodent studies, sulindac does not prevent all polyp growth in FAP patients. A fraction of polyps continue to grow during treatment, and all resume growth after the drug is discontinued.

Results of epidemiologic studies

At least 15 nonrandomized epidemiological studies show that people who take aspirin for prolonged periods for various reasons have 40–50% lower incidence of adenomatous polyps and colon cancer, and similarly lower death rates from colorectal cancer[2]. The results of these epidemiological studies are remarkably consistent, in spite of differences in design, location, population and motivating hypotheses. However, the only randomized clinical trial that examined the NSAID-colorectal cancer hypothesis was the US Physicians' Health Study (n = 22,000)[7]. Neither invasive colorectal cancers, nor *in situ* cancers and adenomatous and hyperplastic polyps were reduced among the physicians treated with 325 mg of aspirin every other day for 4.7 years, compared to controls. The Physicians' Health Study was by no means conclusively negative with respect to NSAIDs and colorectal cancer, because the randomized treatment lasted less than five years, the trial did not screen systematically for colorectal cancer or polyps at the beginning or end of the study, the results were based on few cases, and the aspirin dose was lower than in the experimental and epidemiological studies. However, neither did this well-known trial provide randomized proof of efficacy.

What we don't know

Because of the potentially serious toxic effects of long-term NSAIDs, we need more than rigorous proof that NSAIDs inhibit

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colorectal cancer or adenomatous polyps in the general population. We also need information about the lowest effective dose, the optimal duration and drug, the cellular and enzymatic targets, the contraindications to NSAID use, and proof that at a certain dose the balance of benefits over risks is favorable in defined populations.

Summary

NSAIDs appear to offer much promise against colorectal cancer, yet this potential remains unproven. At present, there is not sufficient evidence to recommend aspirin for the prevention or treatment of cancer[8]. However, there is reason to believe that NSAIDs may have an important role in cancer prevention or treatment if we have the patience to define it.

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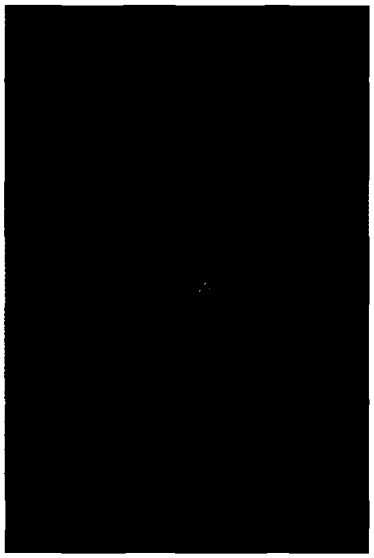
Green fluorescent protein – 'paint in a can'

A typical art store tempts us with row upon row of brillant paints, each with a distinct color that is ours to select. Just imagine the possibilities if a similar assortment of hues was available to illuminate the nooks, crannies and organelles of intracellular space or to serve as differently colored reporters to simultaneously monitor the output of a host of different genes.



A model of wild-type green fluorescent protein based on a structure solved by F. Yang, L. Moss and G. Phillips. Illustration produced by T.D. Romo.

Such choices may soon be ours, according to George Phillips, a biophysicist at Rice University (Houston, TX, USA) who leads one of the two research groups that recently determined the molecular structure of green fluorescent protein (GFP). He refers to GFP as 'paint in a can' and believes that mutagenesis of this most unusual protein paint will soon provide many different colors for labeling cells.

The novel GFP is folded into a cylinder composed of 11 strands of β-sheet with a central α-helix. This structure is novel, and Phillips has proposed that it should be called a β-can. The central helix is also unusual: three amino acids in the helix, serine-65, tyrosine-66 and glycine-67, undergo spontaneous cyclization and oxidation to form the fluorophore of the protein. Usually, protein fluorophores are prosthetic groups noncovalently bound to the protein. The formation of the GFP fluorophore from the amino acid backbone makes GFP an especially attractive candidate for mutagenesis. Slight alterations of the environment surrounding the fluorophore, or in the actual amino acids that make up the fluorophore, are expected to provide a family of GFPs that emit a rainbow of different colors.

Phillips and his student, Fan Yang, in collaboration with Dr Larry Moss at the New England Medical Center (Boston, MA, USA), determined the structure of the wild-type GFP [*Nature Biotechnology* (1996) 14, 1246–1251]. A collaboration between the research groups of Dr Roger Tsien (UCSD, La Jolla, CA, USA), Dr James Remington (University of Oregon, Eugene, OR, USA) and Dr Andrew Cubitt (Aurora Biosciences, La Jolla, CA, USA) led to the determination of the 3D structure of the mutant of GFP in which the normal serine in the fluorophore is replaced by a threonine residue. This mutant GFP has a brighter fluorescence than the wild-type protein [*Science* (1996) 273, 1392–1395]. The structures derived by the two groups are essentially identical.

The West Coast group also used site-directed mutagenesis to convert threonine-203, which is hydrogen-bonded to a phenolic group of the fluorophore, to a tyrosine residue. They reasoned that this alteration would perturb the fluorophore, resulting in a shift in its fluorescence properties. Indeed, they found that the mutated protein had a red-shift in both its excitation and emission spectrum, which they claim to be sufficient for differentiation from the original GFP in fluorescence microscopy.