# β-Catenin, Cyclins, and More: New Insights into the Pathogenesis, Treatment, and Prevention of Colon Cancer

**Athanassios Giannis***

For quite a long time it has been believed that the protein β-catenin plays a critical role during pathogenesis of colon cancer.[1] This suspicion was strengthened by the discovery that the intracellular concentration of β-catenin is greatly increased in the intestinal epithelial cells of patients with the hereditary disease adenomatous polyposis coli.[2] This disease is caused by a mutation of the *APC* gene and represents a predisposition for colon cancer. Furthermore, the *APC* gene is mutated in the majority of colon cancer patients and the gene seems to be decisively involved in this type of cancer.[3]

In normal cells, the APC protein conjoins with β-catenin and axin.[4] In this complex, the β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK-3β) and subsequently, after ubiquitination, degraded in the proteasomes.[5] However, in colon cells with a mutated *APC* gene, this degradation is interrupted and, thereby, the level of intracellular β-catenin is raised.

Up to now, β-catenin has been known to be an important intracellular component, which enables the connection of the cadherin class adhesion molecules (E-, N-, and P-cadherins) with the actin cytoskeleton and, therewith, enables cell–cell adhesion. Also known, but less well studied, was the fact that β-catenin induces the expression of certain genes after nuclear translocation. These genes are, along with others, responsible for cell division and proliferation. However, the mechanism of this remained a mystery.

The situation changed in 1996 when it was found that β-catenin acts as an transcriptional activator after its complexation with DNA-binding proteins of the T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) families.[6] The understanding of this background and the fact that the APC protein keeps the level of intracellular β-catenin low was evidence of the importance of the *APC* gene as a decisive negative regulator of the Wnt signaling pathway,[7] whose excessive activation leads to cancer in humans.

Despite this progress, the nature of the genes which are activated by the β-catenin/TCF complex remained undiscovered. At the beginning of 1999, two research groups independently reported a new breakthrough discovery.[8] Due to the fact that the protein cyclin D1 is an important regulator of the progression of the cell cycle, many research efforts were focused on it as a possible target of β-catenin/TCF. It was also known that cyclin D1 is overexpressed in a significant number of colon carcinomas.[9, 10] Interestingly, the inhibition of cyclin D1 expression by antisense oligodeoxynucleotides abolished the growth of certain colon cancer cells.[11] Investigations in the group of Ben-Ze'ev in Israel showed that the β-catenin/TCF complex stimulates the expression of cyclin D1 in SW-480 colon cancer cells by binding at the cyclin D1 promotor.[8a] Transfection of this cancer cell line with the wild-type *APC* gene leads, by the stimulation of the degradation of β-catenin, to a decrease of free β-catenin and, thereby, to the reduction of cyclin D1 expression. The same result was obtained by overexpression of the cytoplasmatic tail of N-cadherin. The reason is most probably the interception of free β-catenin by the cadherin, which finally leads to reduction of β-catenin/TCF complex concentration. Similar results were found by the group of McCormick in the USA.[8b] Additionally, the authors showed that the oncoprotein Valin-12-p21$^{ras}$ enhances the β-catenin-mediated cyclin D1 expression with the support of the Ets-family transcription factors.[12]

All of these findings clearly show that β-catenin is an oncoprotein. The APC protein is an important physiological antagonist of β-catenin and, thereby, an antagonist of the Wnt signal transduction pathway. When lacking extracellular stimuli, the APC protein promotes the degradation of β-catenin and supports the migration of colorectal epithelial cells throughout the column of the Lieberkühn crypts. The stabilization of β-catenin in the presence of the Wnt signal is probably caused by the inhibition of GSK-3β. This finally results in the suppression of apoptosis and stimulation of proliferation (Figure 1).[1]

What are the implications of these new insights for the development of new colon cancer therapies? One possibility could be the overexpression of the cytoplasmatic cadherin tail which would intercept the free β-catenin. The consequences of this interception concept have to be proven with a suitable in vivo model. The *Min* mouse[13] is an appropriate test system for human adenomatous polyposis coli, because it has a mutated *APC* gene and bears a similar syndrome.

Furthermore, the effect of cyclin D1 could be restrained with suitable inhibitors, such as the synthetic flavonoid

[*] Prof. Dr. A. Giannis
Institut für Organische Chemie der Universität
Richard-Willstätter-Allee 2, 76128 Karlsruhe (Germany)
Fax: (+49) 721-608-7652
E-mail: giannis@ochhades.chemie.uni-karlsruhe.de

  1433-7851/00/3913-2261 $ 17.50+.50/0 

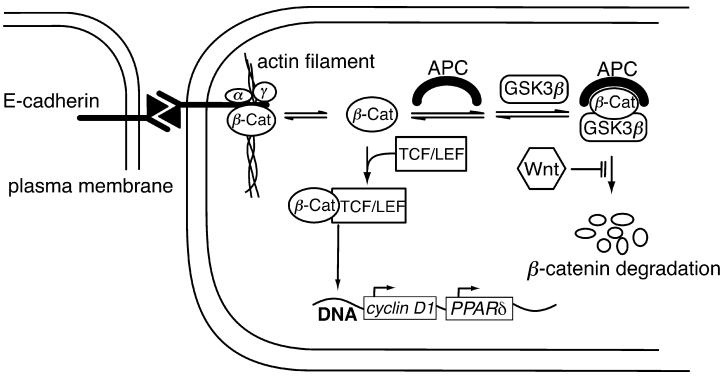
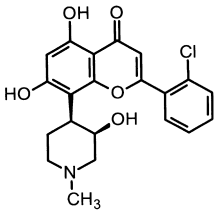


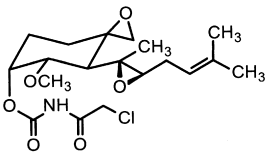

Figure 1. $\beta$-Catenin has a dual function: On one hand, it connects the adhesion molecules of the cadherin class with the actin filament of the cytoskeleton. On the other hand, it activates, in the form of a complex with the transcription factor TCF/LEF, the expression of certain genes, such as cyclin D1 and PPAR$\delta$. Thereby, proliferation is stimulated and/or apoptosis is inhibited. The effect of the $\beta$-catenin is terminated by its proteasomal degradation. For this purpose the proteins APC, GSK-3$\beta$, and axin (not shown) are necessary. In the hereditary disease adenomatous polyposis coli, as well as in many cases of colon cancer, the degradation of $\beta$-catenin is disturbed, due to a mutation of the *APC* gene. This results in a rise of the intracellular level of $\beta$-catenin. Activation of the Wnt signaling transduction pathway enhances the stability of the APC/$\beta$-Cat/GSK-3$\beta$ complex and leads to the same result.

derivative flavopyridol (**1**). Flavopyridol is already in clinical trials and seems to be a promising candidate.[14] It is not only a potent inhibitor for cyclin D1-dependent kinase 4 (cdk4), but also for other kinases which are required for the progression of the cell cycle, like cdk1, cdk2, and cdk7. Interestingly, flavopyridol strongly reduces the expression of cyclin D1. [15]



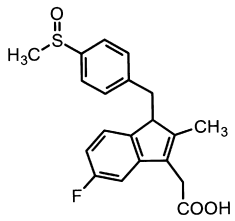
**1**

Regarding the involvement of Ras proteins and Ets transcription factors in cyclin D1 expression, the use of Ras farnesylation inhibitors[16], in combination with inhibitors of the Ets biosynthesis, seems to be promising for the treatment of colon cancer. Fumagillin and its synthetic analogue TNP-470 (**2**) seem to be suitable, because both are not only potent antiangiogenic agents but also inhibitors of Ets-1 expression.[17]



**2**

Further possibilities arise from the reports of Kinzler et al. in the USA. This group recently identified the peroxisome proliferator activated receptor $\delta$ (PPAR$\delta$) as a new target of the $\beta$-catenin/TCF complex.[18] PPAR$\delta$ is a member of the ligand-dependent nuclear receptor superfamily.[19] This family regulates the expression of their target genes by binding the so-called PPAR response element (PPRE) and, thereby, the family controls different aspects of growth, development, and homeostasis. Different polyunsaturated fatty acids and eicosanoid metabolites (such as prostaglandins) were identified as ligands of the PPARs. Kinzler et al. showed that nonsteroidal antiinflammatory drugs (NSAIDs), as sulindac (**3**), inhibit tumor genesis and tumor growth in two distinct ways. On one hand, they inhibit cyclooxygenases (COX-1 as well as the inducible COX-2), which results in the reduction of the formation of prostaglandins. The latter are known to promote the development of colon cancer through paracrine and/or autocrine mechanisms.[20] On the other hand, sulindac can bind to PPAR$\delta$ and disrupt its DNA binding ability. The sulindac sulfone, which is not an inhibitor of COX, is able to suppress the $\beta$-catenin-mediated PPAR$\delta$ activity.[18] Also, in this case, the "good" APC protein acts as an antagonist of the PPAR$\delta$ expression, by stimulating the degradation of $\beta$-catenin.



**3**

Selective inhibitors of COX-2 were successfully used for chemopreventation of colon cancer in different studies.[21] The results of Kinzler et al. present a plausible explanation for the molecular mechanisms. They indicate that COX inhibitors, which are also potent inhibitors of PPAR$\delta$, may be more useful.[22] In this context, it seems important to point out that cyclooxygenase inhibitors show an additional favorable property for tumor therapy—they block the formation of new blood vessels (angiogenesis).[23]

In summary, one can say that, in the last few years, decisive progress has been made in the understanding of the molecular basis for the pathogenesis of colon cancer. The optimism concerning the development of new efficient agents for prevention, as well as treatment, of this disease is justified. It is an exciting question whether these drugs will simply be aspirin derivatives in the end.[24]


[1] M. Peifer, *Science* **1996**, *272*, 974 – 975.
[2] P. J. Morin, A. B. Sparks, V. Korinek, N. Barker, B. Vogelstein, K. W. Kinzler, *Science* **1997**, *275*, 1787 – 1790.
[3] K. W. Kinzler, B. Vogelstein, *Cell* **1996**, *87*, 159 – 170.
[4] a) L. Zeng, F. Fagotto, T. Zhang, W. Hsu, T. J. Vasicek, W. L. Perry, J. J. Lee, S. M. Tilghman, B. M. Gumbiner, F. Costantini, *Cell* **1997**, *90*, 181 – 192; b) J. Behrens, B. A. Jerchow, M. Würtele, J. Grimm, C. Asbrand, R. Wirtz, M. Kühl, D. Wedlich, W. Birchmeier, *Science* **1998**, *280*, 596 – 599.
[5] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, *EMBO J* **1997**, *16*, 3797 – 3804.
[6] a) J. Behrens, J. P. von Kries, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedl, W. Birchmeier, *Nature* **1996**, *382*, 638 – 642; b) M. Molenaar, M. van de Wetering, M. Oosterwegel, J. Peterson-Maduro, S. Godsave, V. Korinek, J. Roose, O. Destree, H. Clevers, *Cell* **1996**, *86*, 391 – 399.
[7] M. Peifer, *Nature* **1999**, *400*, 213 – 215.
[8] a) M. Shtutman, J. Zhurinsky, I. Simcha, C. Albanese, M. D'Amico, R. Pestel, A. Ben-Ze'ev, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5522 – 5527; b) O. Tetsu, F. McCormick, *Nature* **1999**, *398*, 422 – 426.
[9] J. Bartkova, J. Lukas, M. Strauss, J. Bartek, *Int. J. Cancer* **1994**, *58*, 568 – 573.
[10] N. Arber, H. Hibshoosh, S. F. Moss, T. Sutter, Y. Zhang, M. Begg, S. Wang, I. B. Weinstein, P. R. Holt, *Gastroenterology* **1996**, *110*, 669 – 674.
[11] N. Arber, Y. Doki, E. K.-H. Han, A. Scambato, P. Zhou, N. H. Kim, T. Delohery, M. Klein, P. R. Holt, I. B. Weinstein, *Cancer Res.* **1997**, *57*, 1569 – 1574.
[12] K. Inoue, C. J. Sherr, L. H. Shapiro, *J. Biol. Chem.* **1998**, *273*, 29188 – 29194.


  1433-7851/00/3913-2262 $ 17.50+.50/0 

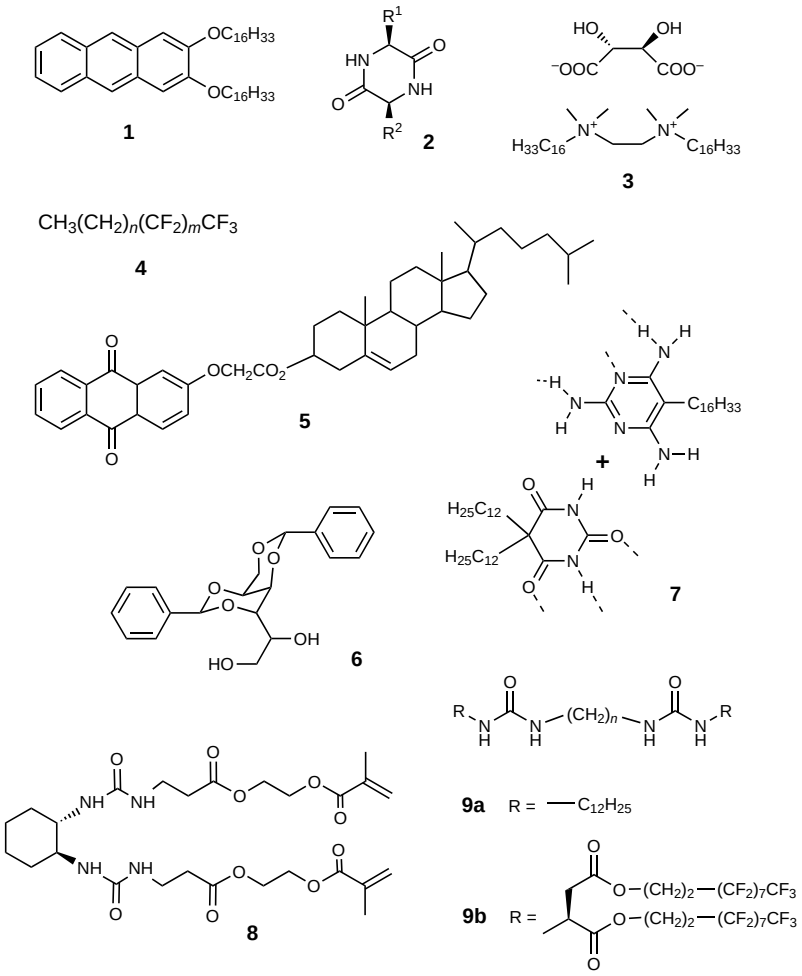
[13] W. F. Dove, K. A. Gould, C. Luongo, A. R. Moser, A. R. Shoemaker, *Cancer Surv.* **1995**, *25*, 335 – 355.
[14] a) H. H. Sedlacek, J. Czech, R. Naik, G. Kaur, P. Worland, M. Losiewicz, B. Parker, B. Carlson, A. Smith, A. Senderowicz, E. Sausville, *Int. J. Oncol*. **1996**, *9*, 1143 – 1168; b) A. J. Kraker, R. N. Booher, *Annu. Rep. Med. Chem*. **1999**, *34*, 247 – 256.
[15] B. Carlson, T. Lahusen, S. Singh, A. Loaiza-Perez, P. J. Worland, R. Pestell, C. Albanese, E. A. Sausville, A. M. Senderowicz, *Cancer Res.* **1999**, *59*, 4634 – 4641.
[16] D. M. Leonhard, *J. Med. Chem*. **1997**, *40*, 2971 – 2990.
[17] N. Wernert, A. Stanjek, S. Kiriakidis, A. Hügel, H. C. Jha, R. Mazitschek, A. Giannis, *Angew. Chem*. **1999**, *111*, 3432 – 3435; *Angew. Chem. Int. Ed.* **1999**, *38*, 3228 – 3231.
[18] T.-C. He, T. A. Chan, B. Vogelstein, K. W. Kinzler, *Cell* **1999**, *99*, 335 – 345.
[19] D. J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, *Cell* **1995**, *83*, 835 – 839.
[20] S. M. Prescott, R. M. White, *Cell* **1996**, *87*, 783 – 786.
[21] D. J. E. Elder, C. Paraskeva, *Nat. Med*. **1998**, *4*, 392 – 393.
[22] G. A. Piazza, D. S. Alberts, L. J. Hixson, N. S. Paranka, H. Li, T. Finn, C. Bogert, J. M. Guillen, K. Brendel, P. H. Gross, *Cancer Res.* **1997**, *57*, 2909 – 2915.
[23] M. K. Jones, H. Wang, B. M. Peskar, E. Levin, R. M. Itani, I. J. Sarfeh, A. S. Tarnawski, *Nat. Med*. **1999**, *5*, 1418 – 1423.
[24] S. J. Shiff, B. Rigas, *Nat. Med*. **1999**, 5, 1348 – 1349.

# New Functional Materials Based on Self-Assembling Organogels: From Serendipity towards Design**

**Jan H. van Esch* and Ben L. Feringa**

Everyone knows what a gel is, but from a scientific point of view the term gel encompasses chemically very diverse systems. Gel systems formed by, for instance, dilute solutions of polymers, proteins, and inorganic substances like silica or clays in water and organic solvents have been well studied and are widely used in, for example, photographic, cosmetics, food, and petroleum industries.[1] In recent years there has been a rapidly growing interest in low molecular weight gelling agents, which is motivated not only by the many potential applications of gels, but also by the fact that these systems exhibit striking properties with respect to self-assembly phenomena (Scheme 1).[2, 3]

These "organogels" have in common with other gel systems that the gelling agent forms a continuous three-dimensional entangled network in the solvent, thereby preventing the liquid from flowing.[4] In contrast to their macromolecular and inorganic counterparts, the network structure formed by low molecular weight organogelators is held together solely by noncovalent forces, including hydrogen bonding, π stacking, and solvophobic effects (Figure 1). The self-




Scheme 1. Examples of low molecular weight gelling agents, illustrating the enormous structural diversity among organogelators.

[*] Dr. J. H. van Esch, Prof. Dr. B. L. Feringa
Laboratory for Organic Chemistry
Stratingh Institute, University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
Fax: (+31) 50-3634296
E-mail: esch@chem.rug.nl

[**] The Royal Netherlands Academy of Science is gratefully acknowledged for a fellowship for J.H.v.E.

  1433-7851/00/3913-2263 $ 17.50+.50/0 