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Commentary

Protein methylation at the C-terminus of mammalian isoprenylated proteins has been implicated in membrane attachment, protein-protein interactions, and protein stability. A new paper describes surprising results: in the absence of methylation some target proteins have increased stability, whereas others have decreased stability. The decreased stability of the RhoA protein is correlated with an increased resistance to Ras-dependent transformation and suggests the basis for the development of a new approach to antitumor therapy .

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Fighting cancer by disrupting C-terminal methylation of signaling proteins

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Protein methylation at the C-terminus of mammalian isoprenylated proteins has been implicated in membrane attachment, protein-protein interactions, and protein stability. A new paper describes surprising results: in the absence of methylation some target proteins have increased stability, whereas others have decreased stability. The decreased stability of the RhoA protein is correlated with an increased resistance to Ras-dependent transformation and suggests the basis for the development of a new approach to antitumor therapy (see the related article beginning on page 539).

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Covalent modification of proteins facilitates a tremendous expansion of their functional potential. Some modifications serve to enlarge the chemical diversity of proteins beyond that provided by the 20 standard amino acids utilized in protein synthesis, providing the new shapes and reactive groups that allow new types of binding and catalytic interactions. Other modifications, often reversible, serve to modulate protein function. Among the large

number of reactions that modify intracellular eucaryotic proteins are three sequential enzymatic steps that recognize proteins synthesized with a C-terminal CAAX tetrapeptide motif, where C is a cysteine residue, A is generally an aliphatic residue, and X can be a variety of residues (1–3). Such proteins are initially lipidated in a reaction that adds either a 15-carbon farnesyl or a 20-carbon geranylgeranyl group to the sulfur atom of the cysteine residue. This step is followed by the removal of the three AAX amino acids generating a C-terminal isoprenylcysteine residue. The third step is a potentially reversible methyl esterification reaction of the newly exposed C-terminal cysteinyl carboxyl group by a membrane-bound enzyme of the endoplasmic reticulum (Figure 1). Many proteins

are modified in this way, including some nuclear lamins, a cGMP phosphodiesterase, and several small G-proteins involved in cell signaling, such as the Ras and Rho proteins. The importance of Ras, especially the activated oncogenic forms, has led to a large interest in this pathway in terms of the possibility of controlling human cancers.

These reactions can provide the modified protein increased membrane attachment with the newly formed hydrophobic C-terminus (4), directed binding to signaling partners via the isoprenyl and methyl groups (5, 6), and protection against proteolytic degradation (7, 8). In this issue of the *JCI*, Bergo et al. provide new insight into these functions and have identified new targets for the development of anticancer drugs (9). In this study, they developed transgenic mice (and fibroblast cell lines derived from these mice) where the expression of the *Icmt* gene encoding isoprenylcysteine carboxyl methyltransferase (*Icmt*) can be controlled by the *Cre-loxP* system. There are two striking results from this study. First, the authors show that loss of the methyltransferase can have different effects in different methyl-accepting proteins. For example, they show that Ras proteins are stabilized but the RhoA protein is destabilized. Second, they show that the decreased levels of RhoA in the absence of methylation results in increased levels of p21^{Cip1} and the inhibition of the cell cycle. The authors thus present a case for anticancer therapeutic approaches based on inhibiting *Icmt*.

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Nonstandard abbreviations used: isoprenylcysteine carboxyl methyltransferase (*Icmt*); S-adenosylhomocysteine (AdoHcy).

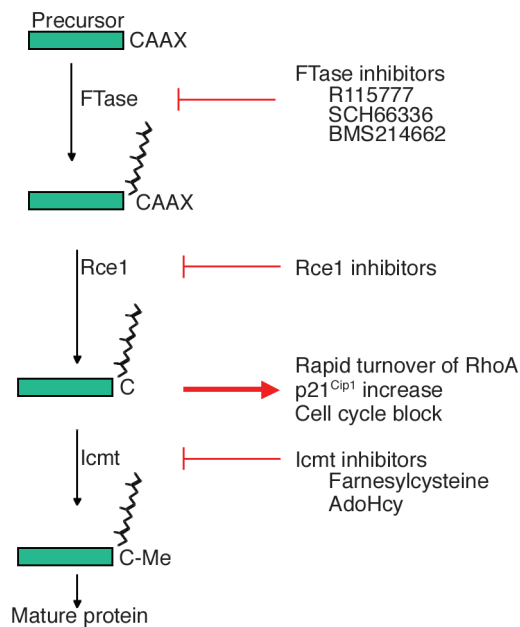


Figure 1

Modification pathway of proteins for isoprenylation/methylation and effects of inhibitors of each enzymatic step. Proteins ending with the CAAX motif undergo farnesylation in the cytosol. Subsequent removal of three C-terminal amino acids and C-terminal methylation are catalyzed by the endoplasmic reticulum-bound enzymes Rce1 and Icmt, respectively. Inhibition of Icmt leads to rapid turnover of RhoA, increase of p21^{Cip1}, and cell cycle block. R115777, SCH66336, and BMS214662 are farnesyltransferase (Ftase) inhibitors, while farnesylcysteine and AdoHcy inhibit Icmt.

Stabilization of isoprenylated proteins by methyl ester formation

Unmethylated isoprenylated cysteine groups at the C-terminus of a protein can be particularly susceptible to proteolytic attack. For example, in yeast strains lacking the *STE14* gene encoding the ortholog of the mammalian *Icmt* gene, a soluble form of the Ras protein accumulates and has the gel mobility of the non-isoprenylated precursor protein rather than the unmethylated protein (7, 10). Unmethylated intracellular yeast a-mating factor is subject to a fivefold higher rate of proteolytic degradation (11). In mammalian cells, inhibition of methylation in a macrophage cell line results in the decrease of the half-life of the RhoA small G-protein from 31 to 12 hours, and of the Cdc42 small G-protein from 15 to 11 hours (8).

Biochemists search for simplicity in understanding metabolic reactions, and it was thus reassuring to see that Bergo et al. found that the level of RhoA was reduced 90 to 95% in *Icmt*-deficient fibroblasts (9), clearly confirming the results of Backlund (8) in a distinct cell culture system. In fact the complete absence of *Icmt* in the fibroblasts led to an even greater reduction in the half-life of RhoA from 22 to 2.8 hours. However, nature often reveals her more complex side, and Bergo et al. found that another isoprenylated signaling protein dis-

played just the opposite behavior! They measured an increase in the half-life of K-Ras from 13.9 hours to 32.5 hours (9). Clearly, there is now no direct relationship between proteolytic stability and the presence of the carboxyl terminal methyl ester.

Loss of methyl esterification by the Icmt enzyme is associated with cell growth inhibition

Bergo et al. found that inhibition of the methyltransferase caused reduced growth and inhibited K-Ras-induced oncogenic transformation (9). While this result is exciting, it is perhaps not totally unexpected, given that K-Ras is a substrate for isoprenylation and methylation. What was surprising, however, was the authors' finding that the effect was apparently not directly associated with the inhibition of K-Ras function itself; Ras-dependent growth factor-induced activation of Erk and Akt was unaffected. It turns out that much of the inhibition of transformation can be laid at the door of unmethylated RhoA, where the reduction in protein level is correlated with an upregulation of the p21^{Cip1} protein that binds cyclins and stops the cell cycle. Furthermore, the effect of *Icmt* deficiency is not limited to K-Ras-induced transformation: transformation induced by an activated (V599E) form of B-Raf was also attenuated by the loss of *Icmt*.

Signaling from the endoplasmic reticulum

The finding by Bergo et al. (9) that K-Ras can signal activation of downstream events — even though they show that K-Ras is not associated with the plasma membrane — is quite intriguing. What could account for this observation? It is possible that a small amount of K-Ras is associated with the plasma membrane and is enough to signal downstream events. It is also possible that a Ras-independent pathway functions to catalyze growth factor-induced activation of Erk and Akt in the absence of *Icmt*. Finally, Bergo et al. (9) suggest the possibility that K-Ras can signal from intracellular locations such as endoplasmic reticulum and Golgi. This idea is particularly interesting, given the recent report that Ras targeted to the intracellular membranes can activate the Erk pathway (12).

Cancer therapeutics

Inhibition of posttranslational modification of signaling proteins provides a promising approach to anticancer therapy. Enzymes catalyzing the three consecutive biochemical reactions on CAAX motif proteins are all potential targets of drug action. Much of the recent work has focused on small-molecule inhibitors of the protein farnesyltransferase that catalyzes the addition of a C-15 isoprenyl group to the cysteine side chain of many CAAX motif-containing proteins (13). Many of these

compounds are competitive inhibitors of farnesyltransferase with respect to its substrates farnesyl pyrophosphate and the CAAX motif (1, 14). In addition, compounds that inhibit farnesyltransferase by chelating zinc, a tightly associated metal that is involved in catalysis, have been identified (15). In preclinical studies, these compounds could inhibit the growth of tumors in mice and even cause regression of Ha-Ras-activated tumors (14). Several inhibitors are currently being evaluated in clinical trials, and beneficial effects have been reported with hematological and solid malignancies (16). However, it has been disappointing that these inhibitors have so far failed to exhibit efficacy against pancreatic tumors where oncogenic forms of Ras are found at high frequency (16). This failure has been attributed to the action of the apparently redundant geranylgeranyltransferase I on K-Ras.

The mixed success with farnesyltransferase inhibitors has led investigators to explore the possibility of targeting the protease Rce1, which removes the AAX residues, as well as the Icmt enzyme. Since only a single enzyme catalyzes each of these reactions (17, 18), this approach may be more effective. It has been shown, for example, that Rce1 inactivation reduces Ras-induced transformation (19). The results of Bergo et al. (9) now provide convincing evidence that targeting Icmt can also be effective in blocking the transforming potential of K-Ras and other isoprenylated proteins.

Present status of inhibitors of Icmt and other methyltransferases

Two approaches have been taken to develop inhibitors of cellular methylation reactions. The first is based on the inhibition of nearly all methyltransferases by the product S-adenosylhomocysteine (AdoHcy) (20). Compounds that increase cellular AdoHcy levels by inactivating AdoHcy hydrolase have both antiviral (21) and antitumor (22) activity. The targets of these drugs are not clear; it is possible that their effects may occur on what has been estimated as more than 300 methyltransferases in the human genome (23) as well as at non-methyltransferase sites (21). But one target may certainly be Icmt. Along those lines, inhibition of Icmt through elevation of AdoHcy levels has been pro-

posed as a mechanism for the antiproliferative effects of methotrexate, a widely used chemotherapeutic agent (24).

A second approach has been to develop inhibitors that would be specific to Icmt (reviewed in refs. 3 and 7). There was initial excitement about the possibility of using derivatives of farnesylcysteine to this end, but this enthusiasm was tempered by the realization that these compounds could block protein-protein interactions based on isoprene group recognition (25). Additionally, incubation of Ha-Ras-transformed rat embryo fibroblasts with farnesylcysteine under conditions that resulted in the 60–70% reduction in Ha-Ras methylation did not significantly affect the growth or transformation of these cells (26). However, given the results of Bergo et al. (9), it now appears worthwhile to resume the search for more effective inhibitors of the Icmt-encoded methyltransferase that may mimic the antiproliferative effect of the knockout of this enzyme in mice. Because the Icmt enzyme is one of a relatively small class of methyltransferases characterized by multiple membrane-spanning segments, it may be possible to develop a new class of inhibitors that take advantage of this particular structure (3).

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