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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Conflict of interest: The authors have declared that no conflict of interest exists.

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

Covalent modification of proteins facilitates a tremendous expansion of their functional potential. Some modications serve to enourage 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 intra- cellular 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 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 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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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 displayed 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 farnesyltrans-
erase 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 cur-
tently being evaluated in clinical trials, and beneficial effects have been report-
ed with hematological and solid malign-
ancies (16). However, it has been dis-
appointing that these inhibitors have so far failed to exhibit efficacy against pan-
creatic tumors where oncogenic forms of Ras are found at high frequency (16). This failure has been attributed to the action of the apparently redundant ger-
anylgeranyltransferase I on K-Ras.

The mixed success with farnesyltrans-
erase inhibitors has led investigators to explore the possibility of targeting the protease Reo1, which removes the AAX residues, as well as the Lcm 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 Reo1 inactiva-
tion reduces Ras-induced transforma-
tion (19). The results of Berger et al. (9) now provide convincing evidence that targeting Lcm can also be effective in blocking the transforming potential of K-Ras and other isoprenylated proteins.

Present status of inhibitors of Lcm and other methyltransferases

Two approaches have been taken to develop inhibitors of cellular methyla-
 tion reactions. The first is based on the inhibition of nearly all methyltrans-
ersases by the product S-adenosylhomocys-
teine (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 Lcm. Along those lines, inhibition of Lcm through elevation of AdoHcy levels has been pro-
posed as a mechanism for the antipro-
liferative effects of methotrexate, a wide-
ly used chemotherapeutic agent (24).

A second approach has been to devel-
op inhibitors that would be specific to Lcm (reviewed in refs. 3 and 7). There was initial excitement about the possi-
Bility of using derivatives of farnesylcys-
teine 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 farnesylesteine under conditions that resulted in the 60–70% reduction in Ha-Ras methyla-
tion did not significantly affect the growth or transformation of these cells (26). However, given the results of Berger et al. (9), it now appears worthwhile to resume the search for more effective inhibitors of the Lcm-encoded methyl-
transferase that may mimic the antipro-
liferative effect of the knockout of this enzyme in mice. Because the Lcm enzyme is one of a relatively small class of methyltransferases characterized by multiple membrane-spanning seg-
ments, it may be possible to develop a new class of inhibitors that take advan-
tage of this particular structure (3).

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