Enzymatic targets for anti-cancer therapy

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Many intracellular proteins, such as the RAS proteins, undergo three posttranslational processing steps at a carboxyl-terminal CAAX motif (Figure 1). First, a farnesyl or geranylgeranyl lipid is attached to the cysteine residue by farnesyltransferase (FTase) or geranylgeranyltransferase type I (GGTase-I). Second, the last three amino acids are cleaved off by RAS converting enzyme (RCE1). Third, the newly exposed isoprenylcysteine is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT). These modifications render the carboxyl terminus of CAAX proteins more hydrophobic, facilitating membrane interactions.

The RAS proteins are involved in the pathogenesis of more than half of all forms of cancer. The processing of the CAAX motif is important for the ability of RAS to transform cells into cancer cells. Our overall objectives are to understand the physiologic and medical importance of the enzymes that carry out the posttranslational processing of CAAX proteins, to define the importance of these enzymatic processing steps in the development of cancer, and to determine if these enzymes are suitable targets for anti-cancer drugs.

Targeting the CAAX processing enzymes in the treatment of RAS-induced cancer

Hyperactive RAS signaling is implicated in the pathogenesis of many human cancers, including lung, colon, pancreatic, and hematopoietic cancer. Increased RAS signaling is commonly caused by acquired mutations that lead to constitutive activation of RAS. However, increased RAS signaling can also be elicited by mutations in genes that encode proteins that interact with the RAS proteins, such as BCR/ABL, FLT3, and the tumor suppressor neurofibromin (NF1). Thus, the RAS proteins and the RAS signaling pathway are attractive anticancer targets.

One strategy to block RAS signaling is to inhibit the enzymes that process the CAAX motif and thereby prevent RAS from reaching its site of action at the inner surface of the plasma membrane.
FTase inhibitors (FTIs) prevent RAS from reaching the plasma membrane, and they have shown promise against a variety of malignancies in preclinical cell culture and mouse models. However, clinical trials in humans have been disappointing. One potential explanation is that several CAAX proteins, including K-RAS (the isoform most commonly mutated in cancer), can be alternately prenylated by GGTase-I in the setting of FTI therapy, thereby retaining a mechanism for proper membrane attachment.

This alternate prenylation pathway, a potential drawback of FTI therapy, has fueled interest in developing GGTase-I inhibitors (GGTIs) and also inhibitors of proteolysis and methylation—posttranslational modifications that are shared by farnesylated and geranylgeranylated CAAX proteins. Thus, GGTIs could potentially be used in combination with FTIs to block the prenylation of K-RAS, and inhibitors of RCE1 or ICMT might block K-RAS activity even if the protein was alternately prenylated. The rationale for inhibiting GGTase-I, RCE1, or ICMT in cancer therapy is bolstered by the fact that other CAAX proteins participate in tumor growth and metastasis.

**Defining the suitability of the CAAX processing enzymes as anti-cancer targets**

Several FTIs and GGTIs have been developed and also some inhibitors of RCE1 and ICMT. However, in some cases compound-specific and off-target effects have made it difficult to decipher their potential utility. In other cases, the first-generation drugs were not specific enough. To be able to establish, with certainty, whether or not the CAAX processing enzymes are viable therapeutic targets, we have to use genetic strategies.

We have developed genetic strategies with genetically modified mice that allow us to switch on cancer in cells and tissues and, at the same time, switch off the expression of each of the four CAAX processing enzymes. Using this approach, we can determine if the absence of these enzymes can prevent the development of cancer.

We recently showed that knocking out GGTase-I, RCE1, and ICMT reduces K-RAS-induced transformation of cells *in vitro* (1–3). We have also shown that while GGTase-I and ICMT deficiency inhibits K-RAS-induced tumor growth *in vivo* (1), RCE1 deficiency actually worsens disease phenotypes in one of our mouse models (4). Accordingly, our experiments now focus on FTase, GGTase-I, and ICMT. We have generated mice with conditional knockout alleles for each of the CAAX processing enzymes and bred new models of lung cancer and leukemia.
In the case of GGTase-I, we generated mice with a rapidly fatal lung cancer and showed that the absence of GGTase-I reduces the development of tumors and improved survival (Figure 2). The tumors that eventually develop in older mice have a more favorable histological grade (1). We also showed that normal non-malignant cells such as fibroblasts, spleen cells, and macrophages are viable in the absence of GGTase-I. We performed similar studies with ICMT and found that knockout of this enzyme also reduced tumor development, but to a lesser degree than the knockout of GGTase-I.

Our studies have shown that GGTase-I and ICMT may be attractive targets for the treatment of cancer induced by mutations in RAS. The fact that several cell types are viable in the absence of these enzymes suggests that the side-effects of drugs that target these enzymes may be relatively low. In the coming year, we will continue to define the impact of inhibiting GGTase-I and ICMT on the development of other forms of cancer, such as leukemia, and also compare the impact of inhibiting GGTase-I and ICMT with the impact of inhibiting FTase.

References

