Review Article
CAAX-box protein, prenylation process and carcinogenesis

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Abstract: CAAX proteins are widely involved in global cellular functions such as proliferation, differentiation, and carcinogenesis. As an important modulator of biological activity, signal transduction via protein prenylation is a crucial step for most CAAX protein functions, particularly for anchoring these CAAX proteins to cellular membrane system. With a better understanding of the molecular mechanisms of signal transduction and intracellular messaging in this process, CAAX protein prenylation may be of particular importance for elucidating the biologic events in carcinogenesis and provide potential approaches of selectively blocking the downstream signal cascade that allows carcinogenesis. Here, we mainly focus on the prenylation process of the clinically important CAAX box proteins, and their potential as a biomarker or preventive/therapeutic target in carcinogenesis.

Key words: CAAX box proteins, prenylation, carcinogenesis, signal transduction, biomarkers

Introduction

CAAX proteins are defined as a group of proteins with a specific amino acid sequence at C-terminal that directs their post translational modification. C is cysteine residue, AA are two aliphatic residues, and X represents any C-terminal amino acid depending on different substrate specificity. The CAAX proteins encompass a wide variety of molecules that include nuclear lamins (intermediate filaments), Ras and a multitude of GTP-binding proteins (G proteins), several protein kinases and phosphatases, etc. Most CAAX proteins are found primarily at the cytoplasmic surface of cellular membranes and are involved in a tremendous number of cellular signaling processes and regulatory events that play various roles in cell biological functions. These activity include cell proliferation, differentiation, nuclear stability, embryogenesis, spermatogenesis, metabolism, and apoptosis [1]. The proteins that have CAAX box at the end of the C-terminal always need a prenylation process before the proteins are sent to plasma membrane or nuclear membrane and exert different functions.

Protein prenylation is post-translational lipid modification process of adding of either farnesyl (15-carbon) or more commonly geranylgeranyl (20-carbon) isoprenoids to cysteine residues of the CAAX box at or near the C terminus of intracellular proteins. This process is critical for proper function of many proteins, particularly for anchoring the proteins to the plasma and nuclear membranes. The prenylation process of the CAAX proteins includes 3 steps: polyisoprenylation, proteolysis, and carboxyl methylation. The first crucial step is that an isoprenoid lipid is attached to the CAAX box by prenyltransferase, for example, farnesyltransferase (FTase) or geranylgeranyltransferase type I (GGTase-I). FTase and GGTase-I recognize CAAX box in protein, and then add the 15-carbon isoprenoid farnesyl pyrophosphate by FTase or the 20-carbon isoprenoid by GGTase-I to the cysteine residue on CAAX box. The 15-carbon isoprenoid farnesyl pyrophosphate (FPP) or the 20-carbon isoprenoid geranyl-geranyl pyrophosphate is a product of mevalonate (MVA) metabolic pathway. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase),
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FTase and GGTase-I are the key enzymes of the mevalonate pathway. The regulation of these key enzymes can significantly affect protein prenylation process. In this review, we mainly focus on the biologically important CAAX box proteins, the role of the prenylation process in carcinogenesis, and the key enzymes involved in the prenylation process as potential targets in cancer prevention and treatment.

Category of CAAX-box proteins

So far, more than 50 CAAX proteins from a wide range of families have been identified. The biologically important CAAX proteins include the G-protein family, nuclear lamin, protein tyrosine phosphatase, GBP Family, battenin family, heat shock protein, proteins with DEAD/H (Asp-Glu-Ala-Asp/His) box, Ser/Thr protein kinase family, GPRK subfamily, PXF/PEX19 family, paralemmin, and inositol-1,4,5-triphosphate 5-phosphatase [1]. These proteins could be further classified into G-protein superfamily, nuclear and intracellular membrane proteins based on their final intracellular locations. A summary of the important CAAX proteins are listed in Table 1.

Table 1. Common human proteins with Carboxyl-terminal membrane-targeting sequence elements

<table>
<thead>
<tr>
<th>Category of CAAX proteins</th>
<th>Proteins</th>
</tr>
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<tbody>
<tr>
<td>G proteins</td>
<td>H-Ras [81], K-Ras4B [15], RhoA [15], RhoB [15], RhoC [15], Rac1/1b, Rac2, Rac3, RhoG [82], Rnd1, Rnd2, Rnd3/RhoE, Cdc42 [83], TC10/RhoQ, TCL, RhoD, Rif/RhoF, RhoH/TF [82], Wrch-1 [83], Chp/Wrch-2 [84], RhoB2/DB2c, Rap1b, Rap2b [85]</td>
</tr>
<tr>
<td>Nuclear surface membrane proteins</td>
<td>prelamin A [86], lamin B1, and lamin B2 [87]</td>
</tr>
<tr>
<td>Intracellular membrane proteins</td>
<td>HsPXF [88], Cenp-F, Cenp-E [25], Pharbin [89], HDJ2 [90]</td>
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RAS proteins and G-protein superfamily

The G-protein superfamily is the most important category of human CAAX proteins. Many G-proteins, such as Ras, Rho, Rac, and CDC42, are located at the plasma membrane and endomembranes. The G-protein superfamily is actively involved in many important cellular signaling pathways and plays an important role in carcinogenesis. As one of the most important G proteins, Ras proteins have a well-established role in carcinogenesis. Ras proteins function as a signal switch that control growth signals from cell surface receptors to nuclear transcription factors. Human cancer studies show that gene mutational activation of the Ras subfamily (K-ras, N-ras and H-ras) occurs in ~20% of human cancers. K-ras gene mutation frequently occurs in pancreatic and colorectal adenocarcinoma [2-6]. N-ras mutation has been reported in melanoma, hepatocellular cancer, myelodysplastic syndrome and acute myelogenous leukemia [7-9]. Several human cancers such as thyroid follicular and papillary carcinoma, bladder cancer and renal cell cancer harbor H-ras mutations [10, 11]. These mutations stabilize Ras in a constitutively active GTP-bound conformation, and constantly activate the downstream cellular proliferation process. In addition to the common Ras mutation, alterations in factors that lie upstream of Ras pathway such as the growth factor receptor tyrosine kinases (RTKs), or downstream of Ras pathway such as BRAF mutation, amplification of p110, AKT2 and deletion of PTEN, also contribute to the deregulated Ras signaling in 30–40% of all human malignancies including thyroid carcinoma, non-small cell lung carcinoma, ovarian and breast cancer [12-14].

Similar to Ras, the majority of the Rho GTPase family is known to undergo similar post-translational modifications on the CAAX box that directs proper subcellular localization required for GTPase function. These locations of the Rho GTPases can vary significantly; some are found predominantly at the plasma membrane (e.g. Rac1), some are associated mainly with endomembranes (e.g. RhoH), and others are associated with endosomes (e.g. RhoD) [15]. Unlike the RAS protein, constitutively active forms of the Rho GTPases as a result of mutations are very rare in tumors. Only RhoH is genetically altered in plasma cell myeloma and non-Hodgkin lymphoma [16].
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has been documented that mutations in the 5’-untranslated region of RhoH independent of chromosomal translocations affect its expression in B-cell lymphomas [17]. Overexpression of other Rho GTPases such as RhoA (in breast, colon, bladder and testicular germ cell tumors), RhoC (in melanoma, pancreatic ductal adenocarcinoma bladder and inflammatory breast cancer), RAC1 and RAC2 (in breast, colon, bladder and head and neck cancers), and CDC42 (in breast cancer) have been reported [18]. Frequently, the increased levels of these GTPases in tumor cells correlate with aggressive histologic features and clinical behavior. Although overexpression alone by no means implies a functional role in the development of the malignancy, the correlation between the increased levels of these GTPases and clinical outcome may suggest an active role of the RhoGTPase in carcinogenesis. Similar to Ras protein, the cycling of Rho protein between inactive (GDP-bound) and active (GTP-bound) conformations is also highly regulated and is necessary for biologic activities such as cytoskeletal remodeling and vesicle transport, many of these being important for malignant transformation.

**Nuclear Laminas**

Nuclear laminas are the major structural components of the nuclear lamina, a filamentous meshwork beneath the inner nuclear membrane. Nuclear lamin provides mechanical stability to the nuclear envelope and controls chromatin organization, DNA replication and anchoring of nuclear pore complexes during cell growth and division [19]. Association of laminas with the inner nuclear membrane is important for their physiological function. The precursor prelamin does not function until a series of posttranslational modifications, including isoprenylation, proteolytic trimming and carboxyl methylation process is completed. The conserved CAAX box at the C-terminal serves as the target of the posttranslational prenylation process. Similar to other prenylation modifications, the CAAX box is firstly recognized by FTase, which adds the farnesyl isoprenoid (15 carbons) to the cysteine residue. Then, farnesylated-proteins converting enzyme 1 (FACE-1), cleaves behind farnesylcysteine to release –AAX. This is followed by subsequent endoproteolytic trimming and carboxyl methylation that significantly increases the hydrophobicity of the C-termini of CAAX-modified proteins. In normal cells, essentially all prelamin A is rapidly converted to mature lamin A so prelamin A is virtually undetectable. However, using protein FTase inhibitors can block the prenylation process, and helps to evaluate the normal function of lamin A [20]. Recent studies show lamin A interacts with DNA directly through the carboxyl-terminal tail or through lamina-associated proteins, which are important for nuclear peripheral positioning and constitutive silencing of heterochromatin [21]. Lamin A also directly interacts and regulates activity of transcription factors, for example retinoblastoma protein (Rb). In addition to promoting subnuclear localization of Rb, Lamin A/C-Rb complex protects Rb from proteasomal degradation [22]. Common mutations involving the normal function of lamin are known to be related to primary laminopathies which includes at least 10 degenerative disorders affecting striated muscle, peripheral nerve, adipose tissue, and cause premature aging with multiple organ degeneration [23].

**Peroxisomal farnesylated protein HsPxF**

Among farnesylated proteins, the peroxisomal farnesylated protein HsPxF is the only one reported so far to be located to peroxisomes, cellular organelles performing metabolic functions. HsPxF proteins encoded by PEX genes are a family of proteins mediating peroxisome biogenesis. HsPxF contains no transmembrane domain, and is loosely associated with the peroxisome membrane rather than being an integral membrane component. For such peroxisomal membrane proteins, the presence of the CAAX box at the C terminal directs the prenylation modification and contributes to subsequent increased affinity to the cytoplasmic surface of peroxisomal membrane. Earlier research indicates mutations in the HsPxF and loss of peroxisome function may be involved in Zellweger syndrome, a human peroxisome biogenesis disorder manifested as progressive neurologic and hepatic dysfunction resulting in early death in infancy [24].

**Other CAAX-box proteins**
Other CAAX-box proteins are involved in diverse biological functions. For example, interferon–induced guanylate-binding protein from the GBP family mediates the anti-proliferative effect of inflammatory cytokines and inhibits endothelial cell migration in the setting of inflammation. Prostacyclin receptor (GPI receptor) is critical in controlling cell proliferation by transducing extracellular growth and proliferation signals to the nucleus. Centromere protein F (Cenp-F) is an important nuclear and kinetochore protein in normal dividing cells. Its prenylation is essential for localization to the nuclear envelope and kinetochores, and for timely progression through G2/M in cell cycle [25]. Prenylation of type I inositol-1,4,5-triphosphate 5-phosphatase facilitates its localization to the cytosol and mitochondrial membrane, and controls the hydrolysis of Ins(1,4,5)P3 during inositol metabolism. Ins(1,4,5)P3 acts as a second messenger for cellular calcium signaling, a pathway essential for many physiological activities [26].

Prenylation is a multistep enzymatic process of adding hydrophobic prenyl moieties to proteins. Prenylation facilitates the attachment of these proteins to the cell membrane. Most of CAAX box proteins do not have a transmembrane domain, thus, the prenylation process is crucial for the function of many signal transduction proteins. The prenylated CAAX box proteins are first anchored to the plasma membrane, and are then transported to the nuclear membrane, mitochondrial membrane or remain as plasma membrane proteins depending on their different structures and cellular functions. The post-translational prenylation process starts a series of molecular signals that control different functions involved in proliferation, differentiation, and oncogenesis.

The prenylation process of the CAAX proteins includes 3 steps: polyisoprenylation, proteolysis, and carboxyl methylation (Figure 1). The prenylation process starts with the attachment of an isoprenoid lipid by protein farnesyltransferase (FTase) or geranylgeranyltransferase type I (GGTase-I) to CAAX box.
FTase and GGTase-I (also called the CAAX prenyltransferases) recognize CAAX box protein, then adding the 15-carbon isoprenoid farnesyl pyrophosphate by FTase or the 20-carbon isoprenoid by GGTase-I to the Cysteine residue on CAAX box. The Prenylation modified Ras proteins are anchored to the endoplasmic reticulum (ER). Rce1 (Ras and a-factor-converting enzyme) and isoprenylcysteine carboxyl methyltransferase (Icmt) remove the AAX and methylate the farnesylcysteine residue. The post-translational modification processes are necessary for downstream effect of Ras proteins.

Steady-state kinetic studies indicate that FTase and GGTase-I recognize CAAX box protein, then adding the 15-carbon isoprenoid farnesyl pyrophosphate (FTase) or the 20-carbon isoprenoid geranyl-geranyl pyrophosphate (GGTase-I) to the Cysteine residue on the CAAX box. In the second step, the CAAX residues are proteolyzed by prenyl protein peptidase RCE1 family on the surface of the ER. The third step adds methyl esterified (OMe) by isoprenylcysteine carboxyl methyltransferase (Icmt) (Figure 2).

The enzymes responsible for isoprenoid addition to proteins are conservative and have been identified in both mammalian systems and lower eukaryotes. Based on the different lipid substrate, protein prenyltransferases can be classified into two functional classes: CAAX prenyltransferases such as farnesyltransferase (FTase) and protein geranylgeranyltransferase type I (GGTase-I); and the Ras geranylgeranyltransferase or protein geranylgeranyltransferase type II (GGTase-II). Here we mainly focus on the CAAX prenyltransferases.

Figure 2. Protein prenylation process is necessary for subcellular localization of mammalian Ras proteins. FTase and GGTase-I recognize CAAX box in protein, and then add the 15-carbon isoprenoid farnesyl pyrophosphate by FTase or the 20-carbon isoprenoid by GGTase-I to the Cysteine residue on CAAX box. The Prenylation modified Ras proteins are anchored to the endoplasmic reticulum (ER). Rce1 (Ras and a-factor-converting enzyme) and isoprenylcysteine carboxyl methyltransferase (Icmt) remove the AAX and methylate the farnesylcysteine residue. The post-translational modification processes are necessary for downstream effect of Ras proteins.
GGTase-I. This may explain why the clinical response is far less than expected in some clinical trials that use FTase inhibitors alone.

**CAAX box proteins as useful biomarkers**

Ras proteins of the G-protein superfamily are constitutionally active in most of malignancy and are known to play a significant oncogenic role in tumorigenesis, thus commonly are used as diagnostic markers. Other CAAX box proteins have also been demonstrated to be associated with cancer. Here, we mainly focus on the nuclear CAAX box proteins and their clinical utility as potential diagnostic or prognostic markers and as the biomarkers for assessing the effects of CAAX prenyltransferase inhibitors.

**Nuclear Lamin**

The expression of nuclear lamin A appears to be linked to cell differentiation in many tumors. It is speculated that lamin A may have a higher affinity for chromatin than type B lamin, thus it may facilitate differential gene expression. Nuclear lamins are differentially expressed in tissues with a variable degree of differentiation and maturation. For example, absence of expression of nuclear lamin A is often associated with embryonal carcinoma, while the expression of lamin A is more common in differentiated non-seminomas. The different staining pattern may help with evaluating the percentage of embryonal carcinoma in non-seminomatous testicular germ cell tumors as a prognostic marker [27]. Nuclear lamin expression pattern was also investigated in normal skin and basal cell carcinoma. Absence of lamin A was correlated with rapid tumor growth, while absence of lamin C was associated with slow growth within the tumor [28]. Similar observations in the expression of lamin A have also been reported in lung cancer. Lamin A expression has been reported in the fast growing small cell lung carcinomas but not in non-small cell lung carcinomas [29].

**Nuclear centromere proteins**

Nuclear centromere protein F (Cenp-F) has a CAAX box and the farnesylation process is necessary for CenpF function at the G2/M transition. Cenp-F gene amplification and overexpression is observed in many malignancies including head and neck squamous cell carcinoma, primary breast cancer, astrocytic gliomas and malignant salivary gland tumors [30-32]. CenpF expression correlates with telomerase activity, cyclin E over-expression, c-Myc amplification, and is associated with poor prognosis and chromosomal instability in patients with primary breast cancer. Cenp-F protein expression evaluated by immunohistochemistry shows correlation with aggressive tumor behavior, and high tumor grade and is an independent predictor of worse breast cancer-specific survival [32]. Cenp-F expression has significant correlation with Ki-67 labeling index in primary malignant salivary gland tumor by immunohistochemical staining and has been suggested as a candidate proliferation marker [31].

**CAAX proteins as surrogate biomarkers for Assessment of the effect of CAAX prenyltransferase inhibitors**

FTase and GGPTase-I inhibitors have been developed as useful anticancer agents. For clinical utility, a correlation between the inhibited farnesylation process and cellular growth, survival and transforming activity or clinical response needs to be established in preclinical studies and clinical trials. This is further complicated by the fact the farnesylated proteins can be alternatively geranylgeranylated when treated with FTase inhibitors. Some farnesylated proteins including H-Ras, prelamin A, lamin B and HDJ2 do not have an alternative prenylation process and thus become more achievable targets to evaluate in clinical settings. The co-chaperone protein HDJ-2 and intranuclear protein lamin A are known to have mobility shifts when FTase is inhibited, therefore are appropriate pharmacologic indices after FTase inhibitor treatment in clinical trials [33]. A mobility shift of HDJ-2 and accumulation of prelamin A after FTase inhibitor treatment could be detected by immunoblotting or immunohistochemistry. Immunoassay has been used for monitoring FTase inhibition in clinical settings [33]. The measurements of FTase activity through HDJ-2 or prelamin A prenylation may not always be adequate surrogates for antitumor activity, especially when the degree of FTase inhibition is not sufficient to produce clinical antitumor effects. However, lack of farnesylation inhibition has been observed in a subset of
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**Mevalonate Metabolic Pathway**

```
Acetyl-CoA  
\downarrow  
HMG-CoA  
\downarrow  
HMG-CoA Reductase  
\downarrow  
Mevalonate-5P  
\downarrow  
Farnesyl-PP  
\downarrow  
Geranyl/Geranyl-PP 
```

- **FTase Inhibitor**
- **GGTase inhibitor**

**Protein Prenylation**

Ras, RhoA, Rac1  
\downarrow  
Chromatin Remodeling & Gene Methylation  
\downarrow  
Cancer

- **Lipitor** blocks protein prenylation through inhibiting HMG-CoA reductase and suppresses the formation of downstream isoprenoids FPP and GGPP, which are used as substrates for prenylation.
- Farnesyltransferase (FTase) inhibitors and Geranylgeranyltransferase (GGTase) inhibitors inhibit a spectrum of protein prenylation (including k-ras, nuclear lamin A and HDJ-2) lead to inhibit mutant k-ras and to modulate chromatin and de-methylate key tumor suppressor genes.

**Figure 3.** Molecular events and mechanisms involved in mevalonate metabolic pathways and potential targets for cancer. Shown are the potential agents/inhibitors (in green boxes) that may impair downstream carcinogenesis (yellow boxes). 1) Lipitor blocks protein prenylation through inhibiting HMG-CoA reductase and suppresses the formation of downstream isoprenoids FPP and GGPP, which are used as substrates for prenylation; 2) Farnesyltransferase (FTase) inhibitors and Geranylgeranyltransferase (GGTase) inhibitors inhibit a spectrum of protein prenylation (including k-ras, nuclear lamin A and HDJ-2) lead to inhibit mutant k-ras and to modulate chromatin and de-methylate key tumor suppressor genes.

Therapeutic potentials of CAAX prenyltransferase inhibitors in inhibiting carcinogenesis

It is crucial in the first step of the prenylation process that FTase and GGTase-I recognize the CAAX box in the protein and add either the 15-carbon isoprenoid farnesyl pyrophosphate (FPP) or the 20-carbon isoprenoid by GGTase-I to the Cysteine residue. The 15-carbon isoprenoid farnesyl pyrophosphate or the 20-carbon isoprenoid geranyl-geranyl pyrophosphate are products of the Mevalonate (MVA) metabolic pathway. HMG-CoA reductase is the first rate-limiting enzyme of the mevalonate pathway. Thus, HMG-CoA reductase, FTase and GGTase-I could be potential targets for modulation of the protein prenylation process as a therapeutic approach, as proposed in Figure 3.

**Farnesyltransferase inhibitors (FTI)**

Farnesyltransferase inhibitors are recently discovered novel peptide analogs capable for inhibiting FTase, thus can influence the protein anchorage to the cell membrane and patients following drug therapy in clinical trials, which usually indicates poor clinical response.
subsequent signal transduction. FTase inhibitors have therapeutic potentials in inhibiting oncogenesis, suppressing unwanted cell proliferation and aberrant high signal transduction. During the last decade, several new FTase inhibitors emerged as a new generation of signal transduction inhibitor drugs targeted against the molecular abnormalities in oncogenesis. Small GTPase H, K- and N-Ras proteins are the most frequently mutated oncogenes in human cancer. Over the past decades, strategies targeting interfering Ras function have always been the subjects of searching therapeutic interventions for human cancer. Since identification of the prenylation process for the Ras pathway, prenyltransferase inhibitors have become a logical approach in therapeutic intervention. The enzyme FTase that is required in the first and essential step of the three Ras processing steps has emerged as the most promising target for anticancer drug development. In preclinical studies, FTase inhibitors show ability in inhibiting tumorigenesis in the majority of cell lines derived from human cancer [34]. Evidence supports that inhibition of FTase of Ras proteins can lead to significant experimental antitumor effects in animal models of colon, pancreatic, lung, prostate, bladder, and breast carcinomas and melanoma [35-37].

In addition to directly interfering with signal transduction in tumors with ras mutations, FTase inhibitors also help indirectly by inhibiting angiogenesis [38], inducing apoptosis and repressing cell division by altering microtubule centromere interactions [39, 40]. In addition, the synergistic effectiveness of combination therapy of FTase inhibitors with traditional cytotoxic chemotherapy agents has been proven in cell lines and in experimental animals [41]. Furthermore, recent studies evaluating the effects of FTase inhibitors as a radiosensitizer show these agents can act in synergy or additively with conventional chemotherapy and sensitize tumors in vivo, or tumor cells in vitro, to radiation therapy. The mechanisms by which FTase inhibitors enhance radiosensitization remain elusive. It is known that hypoxic cells are more resistant to radiation. Tumor oxygenation is improved after FTase inhibitor treatment in vivo. It has been postulated the increased radiation-induced apoptosis may be related to increased oxygenation of the tumor cells’ surrounding tissue [42]. These observations indicate that utility of FTase inhibitors may not be limited to tumors with mutated ras, and expand their potential clinical utility as a radiosensitizer in a wide spectrum of human malignancy.

In the past years, great progress has been made in the use of FTase inhibitors. Several small molecules designed as analogs of the CAAX sequence and peptide mimics have been shown to have efficacy in vitro, as well as in mouse models bearing ras-dependent tumors or human xenografts with H-, N-, or K-ras mutations. These molecules are designed to compete mainly with Ras substrate, not FPP substrate. Thus Ras-dependent tumor inhibition could be achieved without interfering with normal signaling [37]. Evidence supports significant experimental antitumor effects of FTase inhibitors in animal models without significant cytotoxicity in normal cells [35-37]. FTase inhibitors have also demonstrated promising activity in preclinical studies [43]. However in clinical trials, the efficacy of FTase inhibitors is far less than expected [1]. One explanation is that inhibiting farnesylation alone is not sufficient; geranylgeranylation might activate Ras and suppress the effect of FTase inhibitors. Combined farnesyl/geranyl transferase inhibitors might achieve better clinical outcomes.

Geranylgeranyltransferase inhibitor (GGTII)

In the first step of the prenylation process of CAAX proteins, geranylgeranyltransferase type I (GGTase-I) modifies Ras-related GTPases with a 20-carbon geranylgeranyl lipid. The potential of GGTase-I as a target for anti-cancer drugs has been investigated, especially considering that partial rescue of cell proliferation could be accomplished through geranylgeranylation in farnesyltransferase inhibitor treatment. Similar to FTase inhibitors, GGTase inhibitors have been shown to arrest human tumor cell proliferation and reduce tumor growth in animals when used in combination with chemotherapeutic agents [44]. Combined farnesyl/geranyl transferase inhibitors have also demonstrated markedly higher levels of apoptosis than achievable by either farnesyltransferase inhibitors or GGTase inhibitors alone [1, 45]. However, in some preclinical studies, GGTase inhibitors have the ability to impair transformation in vitro and
tumor growth in vivo when used alone [45]. Other investigators report that doses of GGTase inhibitors sufficient for inhibition of K-Ras prenylation are lethal to mice when continuously infused [46]. One explanation is that GGTase inhibitors target many proteins that are important for cell viability. These observations indicate that the clinical benefit may be limited by the toxicity associated with GGTase inhibitor treatment.

**Natural inhibitors of protein prenyltransferases**

Several research groups have screened compounds with inhibitory activity against protein prenyltransferases originating from natural sources. Recently, several natural inhibitory compounds have been isolated and identified from fungal (Gliocladium fimbriatum) metabolite gliotoxin and an organosulfur compound from garlic (diallyl disulfide). Gliotoxin is a sulfur-containing compound that inhibits both FTase and GGTase, and has low toxicity and pronounced anti-tumor activity against tumor cell lines derived from lymphoma and breast cancer [47, 48]. Although the mechanism of their action is unclear, it appears the molecular structures of these compounds are important. Their biologic activity is based on a negative interaction with the zinc ion in the active site of prenylating enzymes.

**HMG-CoA reductase inhibitor**

The mevalonate pathway has also become an important target for anti-cancer therapy. Covalent attachment of these Mevalonate (MVA) derived isoprenoid groups, such as 15-carbon isoprenoid farnesyl pyrophosphate (FPP) or the 20-carbon isoprenoid geranylgeranyl pyrophosphate, is a required step of the prenylation process [49, 50]. There are several important enzymes that convert mevalonate to isopentenyl diphosphate (IPP): mevalonate kinase (MK), phosphomevalonate kinase (PMK), mevalonate 5-diphosphate decarboxylase (MDD), and farnesyl pyrophosphate synthase (FPPS). The enzymes in the mevalonate pathway have become targets for a post-translational modification of proteins that contain C-terminal CAAX.

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase) is the first rate-limiting enzyme of the mevalonate pathway. HMG-CoA reductase, as the main regulatory enzyme in the mevalonate metabolic pathway, is involved in the synthesis of cholesterol and the isoprenoid precursors, farnesylpyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP).

HMG-CoA reductase inhibitors (statins such as Lipitor) are widely used drugs for the treatment of hypercholesterolemia and their potential role as chemopreventive drugs has been investigated [41, 51-56]. Through the mevalonate pathway, HMG-CoA reductase inhibitors such as statins inhibit the formation of downstream isoprenoids FPP and GGPP, which are used as substrates for prenylation, then inhibit the membrane targeting and signal transduction of CAAX protein. Increased HMG-CoA reductase activity and elevated mevalonate synthesis have been found in various malignant tumors, including pancreatic carcinoma [57, 58], colorectal carcinoma, lung adenocarcinoma, hepatocellular carcinoma, and leukemia [52, 59-63]. It has been reported that statins such as Lipitor can inhibit cell proliferation and trigger apoptosis of cultured tumor cells derived from many cancers such as acute myelogenous leukemia cells, juvenile myelomonocytic leukemia, breast cancer, pancreatic tumor, renal carcinoma, astrocytoma, neuroblastoma and several other tumors [53, 64-70]. Dimitroulakos et al used cDNA microarray to identify the differentially expressed genes following treatment of squamous cell carcinoma cell lines with Lipitor. Their results suggest that the pronounced Lipitor-induced apoptotic response is mediated through inhibiting mevalonate synthesis and further depletion of the mevalonate metabolites [71]. In addition, Lipitor significantly increases the apoptosis rate induced by chemotherapeutic agents such as 5-fluorouracil (5-FU) or cisplatin in the colon cancer cell lines [65]. Several animal model studies using statins have exhibited actions against chemical carcinogen-induced carcinogenesis in the colon, mammary gland, liver, and lung [72-74].

HMG-CoA reductase inhibitors have also been shown to inhibit the invasive and metastatic properties of cancer cells [53, 75]. Statin-induced reduction of cell migration and invasion is believed to be independent of apoptosis and is more likely to be associated...
with GGPP-dependent reduction of matrix metalloproteinases (MMPs) activity and disruption the organization of the actin fibers [76]. Abnormal expression of MMPs is believed to play an important role in tumor cell invasion and progression in several cancers [77]. These findings suggest the HMG-CoA reductase inhibitors could be used to prevent and reduce tumor invasion.

In recent years, there has been growing interest in using natural or laboratory synthesized substances of low toxicity to prevent cancer or reduce cancer risk. HMG-CoA reductase inhibitor is one such chemopreventive agent. A promising approach to enhance the chemopreventive efficacy of statins and reduce the potential toxicity is to use them in combination with other agent having different modes of action [78, 79]. It has been shown that administration of Lipitor (atorvastatin) in combination with aspirin or celecoxib (COX2 inhibitor) displays a significant synergistic effect on the inhibition of azoxymethane (AOM)-induced rat colon carcinogenesis [78-80].

Conclusion

CAAX proteins are widely involved in global cellular functions such as growth, differentiation, and carcinogenesis. As an important modulator of biologic activity, signal transduction via protein farnesylation or prenylation is a crucial step for most CAAX protein functions. With better understanding of the molecular mechanisms of signal transduction and intracellular messaging in this process, CAAX protein prenylation may be of particular importance for elucidating the biologic events in carcinogenesis and provide potential approaches of selectively blocking the downstream signal cascade that is important for tumorigenesis.

To prevent the prenylation process of the oncogenic forms of many proteins with CAAX motif has emerged as a promising strategy. Over the past decade, pharmaceutical companies have developed several prenyltransferase inhibitors with impressive antitumor effect in cancer cell lines as well as in animal models. Several of the compounds have reached phase III clinical trials. Unfortunately, the efficacy of these agents as single agents against tumors in clinical trials has been much less than expected, especially in solid tumors, though these agents show promising potential in combination with other chemotherapeutic agents. Another promising compound, HMG-CoA reductase inhibitor, has demonstrated pronounced anti-inflammatory and cancer preventive effects in the laboratory as a single agent or in combination with nonsteroidal anti-inflammatory drugs. The most important question is whether the results could be translated into clinical utility especially in terms of improved overall survival and quality of life. The clinical data so far are limited. These results need to be confirmed with ongoing randomized double-blinded clinical trials. These approaches will no doubt provide a solid foundation for defining the roles of targeted treatment and chemoprevention that could benefit patients.

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